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REARING OF HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS L.)

Author (editor)

Ivar Rønnestad (editor)

Requestor

Section

Section for Aquaculture

Project No.

Responsible:

Ingvar Huse

Summary:

This report presents a summary of the status of knowledge of halibut rearing, based on ongoing research at Austevoll Aquaculture station.

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REARING OF HALIBUT

(Hippoglossus hippoglossus L.)

Ivar Rønnestad (ed.)

Fisheries Directorate, Oceanographic Research Institute,

Aquaculture section

Aquaculture Station at Austevoll

5392 Storebø

FOREWORD

In the last few years there has been increasing interest in the rearing of halibut. Many would like to start now with some captured halibut so that they could gain experience when juveniles become available on the market. During this period a number of questions have been raised concerning farming of the fish, selection of feed, tank/pen system, etc.. It is often the experience gained during daily contact with the fish over a longer period which provides the most important information during this phase. Scientific reports on halibut do not normally cover this. To satisfy this need for information, it was decided to issue this report.

It is important to emphasize that we still do not know much about rearing halibut. Some of the knowledge referred to in this report is controversial and in some cases also based on individual observations and experiments. Nevertheless, I have chosen to include such information. In addition, it still remains to solve a number of fundamental problems, such as start-feeding. Since new experience and more knowledge will constantly be gained, this report must not be considered the final methodology in rearing of halibut. It is the intention of the undersigned to update, and possibly expand, the report every year so that those interested can be kept up-to-date on the results of research and experience gained. For that reason,

I am very interested in supplementary, new or diverging findings and experience within all fields related to the rearing of halibut (see list of addresses on page 204).

I would like to thank laboratory assistants Audhild Kårdahl, Per Hammerø Jr. and Svein Jarle Vatnøy who have provided valuable material concerning the daily tending of and behaviour of halibut (foodfish and broodfish). Some personal and, so far, unpublished observations and measurements are also included.

I would also like to thank my co-authors who agreed to provide material and commented on this report. Furthermore, I would like to thank a number of colleagues who have commented on all or parts of the report: Jens Chr. Holm, Knut Senstad, Kristin Hamre, Tor Hjertnes, Øyvind Lie and Karin Boxaspen.

And finally a thank-you is directed to BP-Salar for funding the undersigned for writing and editing.

A considerable amount of background information of a more fundamental and popular science nature, which throws some light on the practical aspects has been included. As the basic knowledge and experience are incorporated, every fish farmer can obtain more in-depth information

as required. Some of this material requires somewhat more knowledge of biological concepts than the practical material. To facilitate reading, a different print type (this) has been selected.

Ivar Rønnestad Storebø, 25 November 1988.

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INTRODUCTION

by

Ingvar Huse

In record time Norway has established itself as a leading aquaculture nation in a European and global sense. Even though many countries in Europe and Asia produce larger amounts and have longer traditions, we in Norway have demonstrated a rare ability to quickly organize a coordinated effort to build up a new industry from nothing.

In the early stages research played a modest role. As the potential for development in the aquaculture industry became more evident to all, it became easier to gain an audience for the necessity of research. Other nations also developed an aquaculture industry, and in attempts to preserve speed and advantage, research has come to be seen as an important input factor. This applies, not least, to the domestication of new species.

Halibut is the most important marine specie which will supplement the salmonids in the variety of products from the Norwegian aquaculture industry. Even though cultivation only got going properly in 1983, we are, today, well on the way to

developing a functioning production line, which is reflected in this manual. The reasons for the rapid progress are that the foundation was laid by the many years of international research on species such as plaice, turbot and cod, and the authorities and industry have provided considerable financial support to the activity. That such support has paid off is evident from the fact that in 1988 Norway was the world's largest producer of turbot juveniles. This was as a direct result of public research and development. This also applies to the production of cod fry.

When, then, can we expect to see halibut making a serious impact in fish farming? As can be seen in the manual, the problems relate primarily to the start-feeding of larvae. We justifiably hope to solve most of these problems in 1989. Tasks such as optimizing and upscaling to industrial scale still remain. To be sure, the juvenile production experiments are running at large scale, but based on experience with other species, industrialization, upscaling and optimizing take longer than one would like to think. It is therefore reasonable to assume that large quantities of juveniles should be available on the market in 1992 and that food fish should reach the market in 1996.

When, then, should potential juvenile producers start their activities? Five to six firms are already operating. They have assumed the risk of being pioneers and, of course, hope to gain the resultant advantage if all goes well. For the more cautious and resource-poor, it is advisable to wait until more definitive methods for the activity become available. However, it is quite obvious that eggs will be a scarcity factor during the starting phase. If you believe, as we do, in halibut having a future as reared fish and wish to participate in this development, then you should ensure a supply of eggs from a good broodfish population.

Best of luck!

THE NATURAL BIOLOGY OF THE HALIBUT

by

Tore Haug,

Jens Chr. Holm,

Ivar Rønnestad

and

Birgitta Norberg.

The halibut, **Hippoglossus hippoglossus L.** (helleflyndre, hellefisk, stor-kveite) is the largest of the flatfish. The latin name means horse tongue and probably refers to the shape of the fish. The Norwegian "kveite" originates from "hveita", that is, white fish, referring to the colour of the meat. The older expressions "Hellefisk" - "Heilagr fisk" (Heilagr = holy) is a name from the Roman Catholic period. It referred to the "kveite" being a holy fish only to be eaten during the months of Lent (Pethon 1985).

The halibut is spread all over of the North Atlantic from New York and the Bay of Biscay in the south to Svalbard in the north. It has a close relative in the Pacific Ocean, the Pacific Halibut. Next to the salmon, the Pacific halibut is the most important specie in the North American West Coast fisheries.

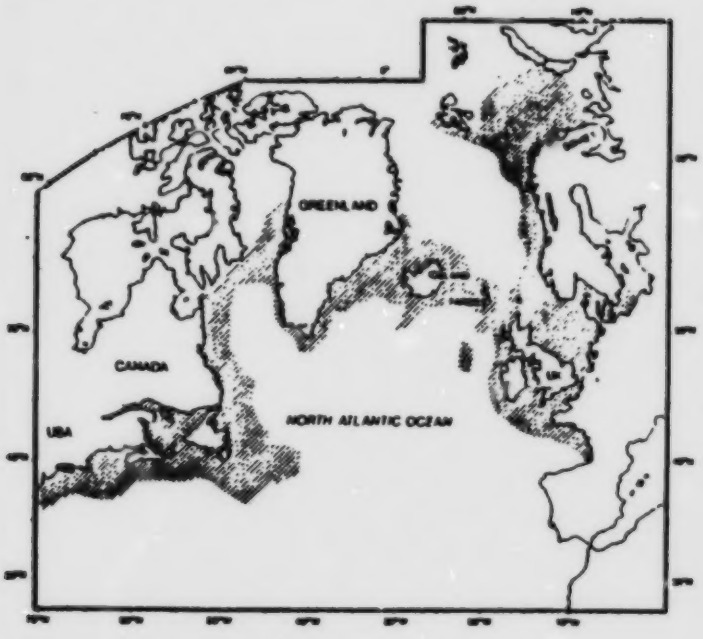

Figure 1. The distribution of halibut in the North Atlantic. (Haug, 1989).

6.

During the summer months, the adult halibut stays on the banks off the Norwegian Coast and in shallower coastal waters. During September - October the halibut migrates towards the spawning grounds. These may be fiords of 300 to 700 metre depth, where the halibut finds deep areas with a clay bottom, or in special areas of up to 1,000 metre depths on the Eggakanten (Hjort, 1905 and Devold 1938). The water in these areas is from 5 to 7 ^{o}C.

The main spawning in the North Norwegian waters takes place during January - February and peaks around the turn of the month (Kjørsvik et al., 1987). In waters outside Vestlandet and near the Faeroe

Islands, the halibut appears to spawn somewhat later in the year, peaking during the months of March - April (Jakupsstova and Haug, 1988).

In trawl and net fisheries on the spawing grounds the catch is dominated by males (Haug and Tjemsland, 1986); Jakupsstova and Haug, 1988). This may indicate that the large females are mostly lying quiet during this period, while the smaller males are more active.

Tagging experiments indicate that the adult halibut returns to the same spawning grounds year after year when the spawning season approaches (Godø and Haug, 1988). After spawning, the halibut disperse over large areas out to the banks or to coastal waters where they hunt for food.

Early development

After spawning, the halibut eggs rise in the water to between 100 and 250 metre depth. Here the temperature is around 5 ^{o}C and salinity at 34 - 34.5 ppt (Haug et al, 1984). At this temperature, the eggs are hatched about 18 days after fertilization. The larvae which emerge from the eggs are about 6 mm long (Blaxter et al, 1983). The yolk sac is large (ca. 90 % of dry weight) and it provides the larvae with nutrients during the first 30 - 40 days.

Very little is known about the natural occurrence and ecology of the larvae since, so far, only one (1) larva has been found in the wild

(Haug et al, 1988). In all probability, they rise slowly up towards the upper layers of water as development progresses. When the yolk sac has been emptied, the halibut larvae have a length of about 11 - 12 mm and are then in the uppermost 10- 50 metres of the water column. Edible plankton can be found in these layers of water.

Ninety days after hatching the larvae have reached a length of 2.5 cm and are almost fully metamorphosed, that is, have acquired the characteristic flatfish features (Berg and Øiestad, 1986). About 4 months after hatching they seek deeper waters, probably to areas about 40 to 50 metres deep. They then measure about 3.5 cm in length (Vedel-Tåning, 1936).

The immature halibut.

We have very little information from nature about the earliest stages of the halibut, from hatching to when the fish has reached the one-year stage. However, from the one to two-year stage we find them in 20 - 50 metres coastal growth areas. Tagging experiments have shown that the small halibut remain in these areas until they reach the age of four to six years (Godø and Haug, 1988). Thereafter they may make long migrations without it being apparent that they select a systematic direction. Only when they have matured sexually do the halibut seek the spawning grounds. It is still unknown whether the selection of spawning ground is dependent on where the individual fishes were born.

Growth and maturation

The most important reason for a halibut becoming big is that it grows old . It takes about 20 years for a female halibut to reach 100 kg and in wild halibut populations the fishes take at least 7 - 8 years to reach a round weight of 10 kg (Jakupsstova and Haug, 1988).

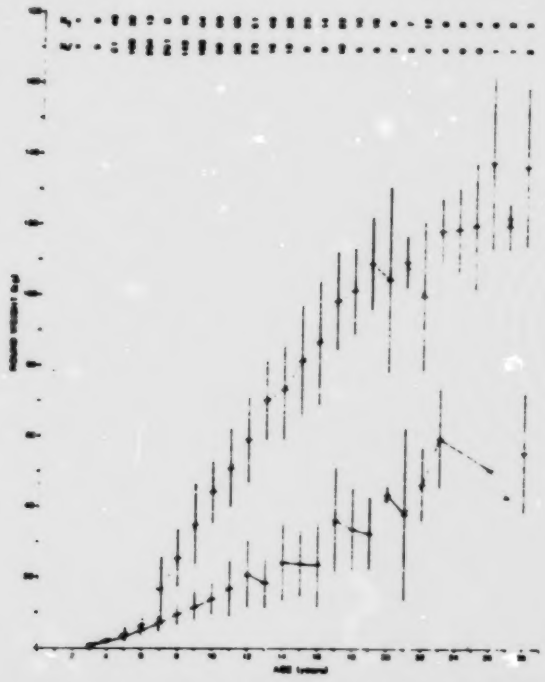

Figure 2. Relationship between age and round weight of halibut caught near the Faeroe Islands. Curve for males is solid, while that for the increase in weight of females is broken (Jakupsstova and Haug, 1988).

However, it has been found that the growth of halibut depends on fish density. Clear signs have been observed that the growth among halibut in North Norway has increased as the population has decreased during the last 25 - 30 years. This has become evident from, among other things, the fishes now maturing at a considerably lower age (7 and 8 years, as opposed to 12 - 13 years in the 1960's. Haug and Tjemsland, 1986).

The females may become considerably larger than the males. It is particularly after sexual maturation that these differences become evident. While the females may be over 330 kg (a fish of 333 kg was reported in Hammerfest) it is rare to find males heavier than 50 kg.

Growth and age at sexual maturity may also vary from area to area. The most rapid growth was observed among halibut from the waters near the Faeroe Islands (Jakupsstova and Haug, 1988). Here the age at maturity was 4.5 years for males and about 7 years for females. The length at sexual maturity is an average of 50 - 55 cm for males and 110 - 115 cm for females. In weight this will mean 1.7 kg for males and 18 kg for females.

8.

Nutrition and reproduction

The halibut is a distinctly predatory fish. Studies carried out near Iceland and the Faeroe Islands have found that the smaller halibut have a varied diet. They eat fishes such as, for example, herring,

haddock, sand eel, as well as squid and crustaceans such as, for example, crab (McIntyre, 1953). The adult halibut eats fish almost exclusively and then particularly redfish. It has been maintained that in some areas redfish can be as important to the halibut as capelin and herring are to the cod.

There are many indications that the halibut eats less during the most intensive spawning period, but the intake of food does not cease completely. During the period approaching spawning the ratio between weight and length ("the condition") decreases to a certain degree among males, but not among females (Haug and Gulliksen, 1988 a). The ability of the female to build up roe without it noticably affecting the edible portion of the fish supports the theory that there are diffences between the sexes in physiology and growth among halibut.

The female halibut starts preparing for the spawning season in July -

August of the previous year. The process is started by a female sex hormone. The halibut will then build up reserves of energy and building blocks in the liver in the form of a protein (vitellogenin). The vitellogenin is altered in the liver by some processes whereby the various nutrients necessary for the larvae are bound to the vitellogenin. The modified protein is transported by the blood to the ovaries (gonads) where it passes through special ducts between the follicle cells surrounding the oocytes (eggs). Vitellogenin is then absorbed in the oocyte. In the oocyte, the vitellogenin is divided prior to storage. During this period when the female fish builds up the gonads, the weight of the oocyte increases several thousand times. In a sexually mature halibut, the gonads constitute between 10 and 15 percent of total body weight. When the eggs are ripe they swell by a massive uptake of water which flows from the blood into the eggs (hydration). (See Haug and Gulliksen, 1988 b). This results in a female fish being capable of producing eggs totalling half its body weight!

We still do not know whether qualitative changes occur in the halibut fillet in connection with spawning. Studies are therefore underway to look at any possible seasonal development or change in these conditions. Since a halibut spawns several times, it is possible that it can be kept through one or several spawning seasons before it is slaughtered.

COLLECTING BROODFISH

by

Ivar Rønnestad

To build up a broodfish population, both males and females are needed. Unfortunately, the difference between males and females is not discernable from external signs. However, there are four other methods of determining the sex of halibut.

1) Measurement of length. Fish longer than 1.3 metres are almost certainly females.

2) Stroking of the fish to see if one gets eggs or milt. This requires a sexually mature fish.

3) Biopsy (tissue sample). This is carried out by passing a soft tube (3 - 4 mm in diameter), mounted on a syringe, into the gonads and sucking out a sample of the tissue.

4) Ultrasound has been successfully used for sex determination of young fish (Mattson and Rønnestad, unpublished).

Fish of more than 60 kg (females over about 1.4 metre) have been found to produce eggs of poor quality in captivity. It is therefore probably of little value to accept these as broodfish.

Capturing.

Despite its size, the halibut is very sensitive to poor treatment. This applies particularly to the vital layer of slime which covers the skin of the fish. The slime layer functions to protect the fish against infections and changes in the balance of liquid. Everything possible must be done to avoid scraping this off. Broken layers of slime cause sores which often lead to the fish not surviving in captivity. Therefore the use of ropes during capturing must be avoided.

The fish must be caught using equipment based on hook (for example, line). All experience has shown that net-caught fish do not survive. Small fish can be caught with a Danish seine. The halibut has no swimming bladder and can be drawn straight up from great depths with no problem (as opposed to, for example, cusk).

The fish must not be lifted by snood and hook. The best method is to lower a board (canvas sail) or the like underneath the fish and lift it on board.

Another method is to punch a hole in the lower jaw (immediately behind the teeth), slip a rope through it and lift by that. Rope must not be used around the tail. It is not

recommended to let the halbut swim in the sea or lie on the bottom with the rope through the lower jaw for several days while awaiting transport to the receiving station.

If the hook holds fast in the mouth, throat or gullet, then the snood must be cut so that a piece about 20 cm long hangs out of the mouth. If the halibut has the hook in the stomach, then, more than likely, it will die after a period of time. If the hook has made a hole in the blind (white) side of the halibut, then this may also develop into large open sores (K.Senstad, personal communication).

10.

Transport

The fish must be kept wet. It is also calmer when kept in darkness. On shorter transports it has been possible to keep the fish alive by pouring buckets of fresh sea water over it, but any transport longer than 30 minutes requires circulating sea water and/or bubbling of oxygen gas (H. Rabben, personal communication).

To avoid scraping off the slime, it is very important that the fish not be placed on a rough coarse surface.

The best method is to place the halibut in a bag with a

flushing hose fastened to the bag at the opening where the head is. The fish should lie in a tank but it may also lie directly on the deck (H. Rabben, pers. comm.).

An ordinary fish well may be used.

When transporting during heavy seas it is important that a system be installed which is adapted to lurching and head seas.

Transfer to broodfish station

All facilities should have a canvas sail available. This should consist of a square metal frame of about 2.3 x 1 metre, with no sharp edges. Inside this frame is lashed a canvas of the same size. This canvas sail is used to transport the halibut from the boat/vehicle, when stroking and at any other time when moving the fish at the facilities. Avoid using landing nets, particularly those with knots.

When the fish is to be transferred to the broodfish facility, it must be checked for sores and parasites. If the fish has any roughness on the skin, that is, there is no slime, then it is quite certain that sores will develop. It is therefore important that a newly caught fish be watched from the beginning. If sores develop on the fish, this most often leads to bacterial infections (Flexibacteria).

Therefore, fish with large sores should, when possible, be isolated and treated with antibiotics (See under Diseases).

During the initial period after capture, the halibut has no appetite. It may take several months before it begins to eat. This depends on the size at time of capture. Large fish have difficulties in starting to eat, at least when kept in small tank systems. In the most difficult cases, force-feeding must be used (see Section on Feed and Feeding). The smaller fish apparently can withstand the stress of capture better and regain their appetite more quickly. Experience has shown that if some fish eat in the tank, this often leads to others in the tank regaining their appetite (P. Hammerø, pers. comm.). This also applies, in general, to a number of species.

Marking of fish

Marking of the fish facilitates identification of the fish in connection with spawning, weight measurements and the like. The need to mark the fish should be evaluated by the individual fish-farmer. Halibut from a size of 130 g (A. Kårdahl, pers. comm.) have been tagged without complications with a Floy tag (see below), but since flounder can be tagged from 10 g, it is likely that this can also be accomplished with halibut (J. Chr. Holm, pers. comm.).

11.

There are several possibilities for marking the fish. From

oceanographic research, a number of various marks/tags and techniques are known. Examples are: "Fingerling tags", burn marks, fin cutting, spaghetti marks, dart tags, Leas tags, carlin tags, freezing marks and Floy anchor tags. The latter two are common on halibut and will be dealt with here.

Floy anchor tags FD-67C).

This type of tag consists of a coloured soft PVC tube about 1.5 mm in diameter and 20 mm long with a stiff nylon thread inside. One end of the nylon thread is slightly thicker and T-shaped (Figure 3). These are fastened to the fish by using, for example, a price tag device (a simple model can be ordered from Storm Price Marking, Oslo).

The tags must be placed all the way out where the fin is fastened to the side of the fish. When this is properly carried out, the T will hook under the fin rays and act as an anchor - hence the name (Figure 3). Care must be taken that the hook has the correct direction when the tag is inserted. The problem with the Floy tag is that the writing, normally in the form of a number imprinted on the PVC tube, is not visible from the surface. However, the tags can be given individual colour codes (two or three colours per tag), so that recognition from the surface is nevertheless possible.

In order to recognize each fish without having to take it out of the water, different tag locations, a number of tags, larger tags and/or tags of different colours can also be used.

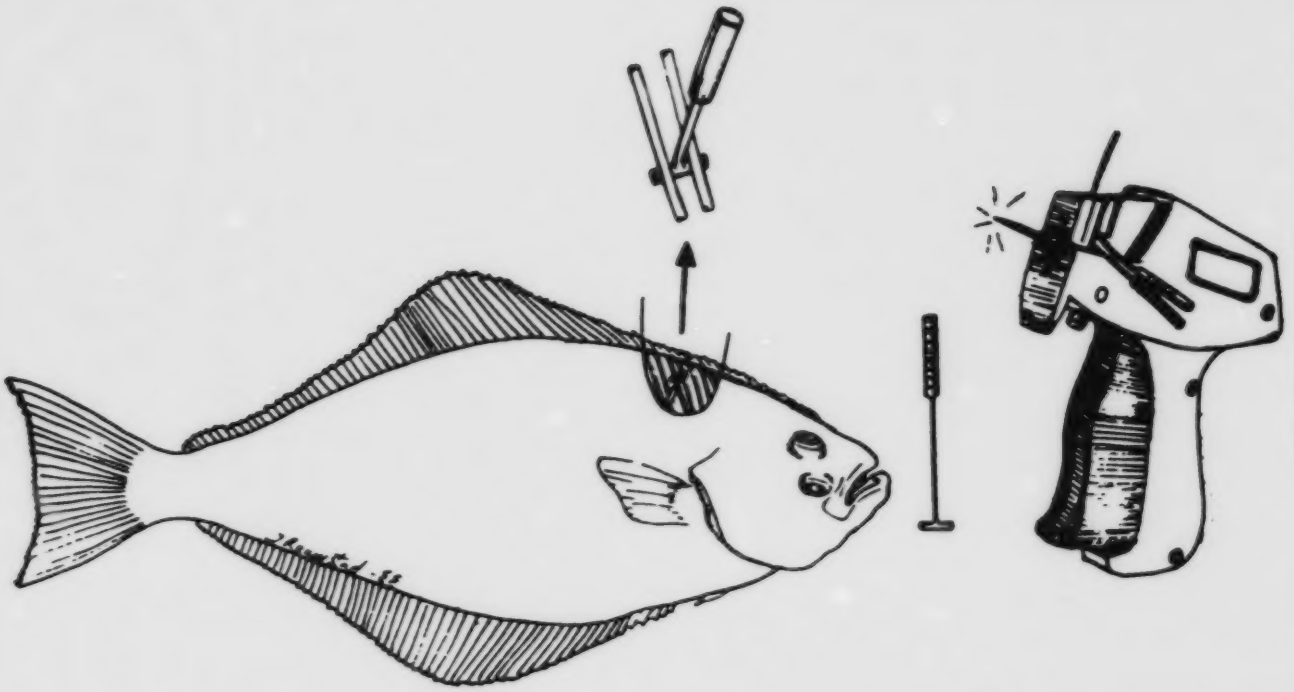

Figure 3. Example of tagging equipment and tagging of halibut.

12.

Large, soft rubber tags like those used on domesticated animals such as pigs, sheep and the like, which can be cut to the appropriate size, can also be used. When using such tags, loose "T-hooks" can be purchased (those used for price marking of clothing). The loose "T-hook" is first stuck through the tag before it is inserted into the fish.

If the halibut is handled carefully then it is not necessary to anesthetize it for marking.

Freeze marking tagging

This method works satisfactorily at one facility, but requires personnel specially trained in reading the tags, and a fair amount of equipment.

The marking is based on the following method: A metal pipe with good conduction properties leads from an insulated container of liquid nitrogen. The various marks, in the form of symbols, are attached to the end of the pipe (Nævdal et al, 1977). The mark will have a surface temperature of about -175 ^{o}C. The fish is pressed against the mark for about 2 - 4 seconds and the symbol remains as a light mark in the skin of the fish. By varying the symbols and location of the mark, a large number of fish can be marked in this manner.

REARING ENVIRONMENT

by

Ivar Rønnestad

Pens/tanks and facilities

Today there are many types of facilities used for rearing halibut. The most common type is the land-based system (round tanks, tanks with longitudinal water flow, concrete pools), but pens, flowing tank systems, lobster pounds and earthen dams are also used. The individual technical solutions are not described here.

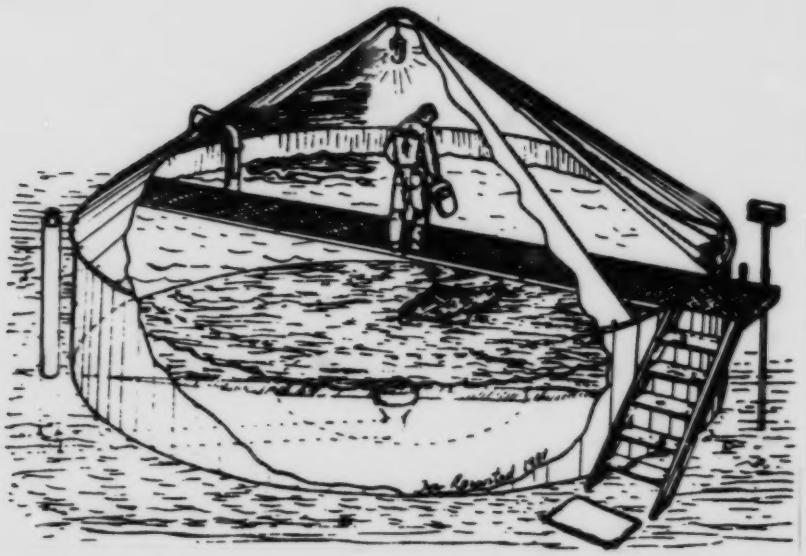

Figure 4. Example of land-based rearing facility for halibut.

In one single case, small halibut were placed together with salmon in a normal pen. This is not recommended for turbot which develops bloody fin edges already after 14 days in a salmon pen (K. Hamre, pers. comm.). It is likely that wear and tear on the fins will also occur to the halibut, but the scope of the problem can be reduced by using fine-meshed netting.

Experiments using a specially built halibut pen with a solid bottom (netting and plywood) have been carried out but without much success in the first trials. It was obviously not easy for the halibut to see the netting and often it collided with it as it headed for open water. These experiments were stopped because of problems with growth on the side netting, and it was difficult to tend to the fish as well (P. Hammerø, pers. comm.).

14.

There appears to be a general concensus that since the halibut is a flatfish and spends large portions of the time on the bottom, a flat bottom should be used regardless of the type of system. Furthermore, from the behaviour of the fish it appears that the comfort of the fish increases with increasing size in both area and volume available to the fish. Round tanks less than five metres in diameter appear to be unsuitable for broodfish. More research is required in this area.

Many of those who come in contact with halibut for the first time believe that it needs high pressure and great depth to survive and function. For financial reasons it is impossible to base the rearing of halibut on advanced and expensive pressurized systems. If the whole production line for halibut cannot be controlled under surface conditions then farming of halibut has no future. Fortunately, it appears that the halibut is quite content at a depth of one metre.

On the other hand, it is possible that problems with gas in the eyes (see "Disease" on page 64) may be caused or aggravated by shallow tanks. This will be investigated further.

Substrata (types of bottom)

Flatfish often dig themselves down and may therefore need a loose bottom. This also applies to halibut even though the light conditions under which this fish lives reduce the significance of camouflage as a defence mechanism against predators. Experiments have shown that reared halibut prefer a sand and gravel bottom to a net and plastic bottom.

It has been found that a group of halibut which were kept in plastic tanks without any extra substrata developed a dark underside. The discolouration was of no significance to growth (Rabben and Huse, 1986, and Senstad and Kvalsund, pers.

comm.). On the other hand, considering the present consumer mentality, such a halibut has a lower market value. Furthermore, halibut kept in a rearing tank with no substrata often develop small puncture bleedings on the underside (A. Kårdahl, pers. comm.).

No research has been carried out in Norway to determine whether the colour of the sand or gravel is of significance to comfort. In Iceland, however, it was found that the halibut prefers dark fine sand to light sand, gravel or a normal tank bottom with no substrata. Halibut caught off Iceland are used to dark sand (lava) as bottom material and this may explain why the Icelandic halibut prefers dark substrata (B. Bjørnsen, pers. comm.).

It is important that the substrata not consist of materials having sharp edges. This applies to gravel in particular. When halibut were first kept at Austevoll Aquaculture Station, normal gravel was used. This resulted in large sores on the underside of the fish due to slime being scraped off, particularly in combination with stroking. Several fish died. The gravel was then exchanged for river gravel which is worn round. The results of these experiments have been good (P. Hammerø, pers. comm.).

In Iceland a layer of sand 5 - 10 cm thick is used as a

substratum. Here, too, the results have been good. The fish digs down into the sand until only the two eyes are visible. The result is that it might be impossible to detect the fish, which, of course, is unfortunate during sickness, but it increases the self-cleansing of the tank. The reasons appear to be that the fish stirs up the waste and dirt which lie in the sand when it digs itself down, so that the sand is continuously being cleaned (B. Bjørnsen, pers. comm.).

15.

In general, a rough substratum increases the possibilities of collecting organic material such as feces and feed. This may make impossible the use of substrata in future high-density food fish rearing facilities. Nevertheless, good bottom circulation and water exchange are important in systems using a substratum.

Covering and water level in tanks

Halibut of all sizes can jump out of a tank if the water level is too high and there are openings through which it can escape. It is not known how high or how far a halibut can jump but heights of 70 cm (K. Senstad, pers. comm.) and distances of about one metre (personal observation) are not uncommon. The reasons the halibut jumps may be many, such as parasites or being chased by a dominant halibut.

This requires that either the tank be covered with netting (see also Lighting) or the water level reduced, so that there is a minimum of 70 cm freeboard. The latter solution increases the cost of the system considerably.

Lighting

At the depths where large halibut normally live it is quite dark; the light only reaches to 100 metres in exceptional cases. The halibut also prefers dark or shady places in rearing facility.

On the other hand, the length of day is probably an important factor for normal growth and sex maturation among halibut. Therefore, some light must come into the facility. If the facility is covered, then it is important to have a natural relationship between light and darkness.

As in the case of turbot, it has been found that halibut can get sunburnt. Therefore, particularly during the summer, care must be taken to ensure that halibut are not exposed to direct sunlight. Too strong light will also increase the growth of algae (both macro and micro algae) in the facility.

The conclusion is therefore that a form of covering (netting, canvas) should be used which lets some natural light through, or artificial lighting in phase with nature if the facility is under cover.

Water Quality.

Intake water

So far, deep-sea water has been used for the land-based rearing facilities which exist for halibut. Deep-sea water has clear advantages over surface water such as, for example, relatively stable temperature, salinity, lower content of biological material (and thereby less growth), less danger of disease, more stable amounts of oxygen, and so on. What can be defined as deep-sea water may be debatable, but when seen in the light of the bloom of deadly algae during the spring and fall of 1988, the intake should be below a depth where it can be assumed that the probability of the presence of algae exists (9 brood halibut at a private facility using surface water died during the last bloom of dinoflagellates (**Gyrodinium aureolum**)). With a little bit of a margin this means 40 metres or deeper. Some people claim there is an advantage in going even deeper. At the College in Bodø, the intake is at a depth of 200 metres. One fish-farmer, who is now in the process of starting up, has stated that he will venture on deep-sea water from as deep as 400 metres.

16.

Temperature

Temperature is one of the most important environmental parameters in connection with fish-farming. In contrast to

surface water, deep-sea water ensures a certain degree of even water temperature the year round. At the Aquaculture Station at Austevoll the water temperature at 50 metres depth is normally between 5 ^{o}C (March) and 11 ^{o}C (October). By using water at a temperature near or lower than the freezing point, mortality occurs in the fish population. During a strong cold snap during the winter of 1987, the water temperature dropped in the broodfish facility at Tromsø Museum to -1 ^{o}C (salt water has a freezing point close -2 ^{o}C). Large numbers of the newly caught broodfish died during this period (T. Haug, pers. comm.).

In Iceland, experience has shown that small immature halibut have both an appetite and increase in weight at +1 ^{o}C. (Bjørn Bjørnsen, pers. comm.). Due to the negative correlation between low temperature and growth, such water temperatures are probably unsuited to food fish farming, even though a certain weight can be achieved. This must be researched further.

In land-based facilities with large surfaces, the water temperature is influenced to a certain extent by the air temperature, sun radiation and wind. This must be taken into

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consideration even if one starts with water of acceptable temperature. This applies especially to large volumes of water where the flow rate is low.

In floating facilities, the water temperature in the tank will also be affected by ambient temperature fluctuations. Where there is a problem with periods of extremely warm or cold surface water, this may alter the water temperature in the system even when using deep-sea water unless: 1) an insulated type of facility is used, or 2) the circulation of intake water is increased.

Salinity.

The halibut is a marine fish, that is, it lives in the ocean all its life in contrast to the salmon. In the wild it prefers high salinity of 33 - 34 ppt. A lower salt content does not appear to cause mortality since the broodfish at Sunndalsøra have been kept at 28 ppt through a whole spawning season (I.Holmefjord, pers. comm.).

During the algae invasion in the Spring of 1988, some small halibut, from two to four kilos, were successfully kept for one week at a salinity of 11 to 15 ppt. This was an emergency case where the salinity was reduced to avoid mortality, but it proved that no acute mortality occurs even at such low salinity over short periods.

Oxygen consumption.

Halibut require less oxygen than do salmon. Original measurements have shown that moderately active halibut of between 1.0 and 4.0 kg consume 0.6 - 1.4 mg O_2/kg/min. at a temperature of 8 ^{o}C (personal measurements, unpublished). 17.

A deciding factor for how much or how little oxygen the fish can tolerate in the water depends on complex physiological properties and not on the normal oxygen consumption. Values for the critical oxygen content/level have still not been worked out. This is normally recorded in the discharge water. For small salmon, minimum values of around 7 ppm (which provides a good margin) are now utilized; for turbot - around 5 ppm. It is likely that the minimum limit for halibut is of the same magnitude as that for turbot.

Water exchange

In halibut rearing is has been normal to use non-recirculating systems. This means that the intake water is pumped directly from the ocean, possibly through a filtration/UV lighting plant, and released into the discharge system afterwards. In such a system, the exchange rate of the water is primarily determined by the need for self-cleansing and, secondly the O_2

consumption. The concentration of ammonia (NH_3) in the water has normally not been of any significance to the requirement to exchange the water in an open sea-water facility.

In general, there are three main criteria for the flow environment in a fish farming unit (Lunde, 87):

- a general flow environment in the whole water volume which is adapted to the size and weight of the individuals.
- Good water exchange in the tank/water which ensures homogeneous quality in all of the water.
- A sufficient bottom flow to provide continuous self-cleansing of the tank bottom.

The fall of 1988 will see some experiments being carried out under the auspices of Norsk Hydroteknisk Laboratorium (NHL) (Norwegian Hydro-technological Laboratory) which should shed some light on the amount of water needed and how the intake and outflow must be designed to maintain a satisfactory water environment in different tanks.

In order to show the amount of water circulation needed in a facility to maintain O_2 level, the following simplified calculation is presented:

Given a tank holding a total of 100 fish, each weighing between 1 and 2 kg.

The water temperature is 6 ^{o}C and the salinity 34 ppm.

The water is fully saturated.

Calculation:

Amounts of O_2 consumed per minute (O_2 consumption is set at 1.4mg/kg/min);

100 kg x 1.4 mg O_2/kg/min = 140 mg O_2/min

100 kg halibut consumes 140 mg O_2/min

Fully saturated water at 6 ^{o}C contains about 10 mg O_2/L (from table).

Water supply:

140 mg O_2/min ÷ 10 mg O_2/l = 14 l/min.

To replace consumed O_2, a supply of 14 l/min of fully saturated water is needed.

18.

The calculations show that the volume of water in the tank is not significant (assuming, among other things, effective exchange) to how much new water has to be added to maintain a constant amount of O_2. On the other hand, it is significant to how quickly the content of O_2 in the tank is reduced when the water is stopped. This will be shown by comparing two tanks of 30,000 l and 5,000 l, respectively. In both cases the water is 100 % saturated before circulation is stopped.

Tank volume = 30,000 l:

To find the total amount of O_2 in the tank (at 6 ^{o}C):

30,000 l x 10 mg O_2/l = 300,000 mg O_2, that is, 300 g O_2

30,000 l contains 300,000 mg O_2

To find out how much O_2 remains in the tank one hour after the water has been shut off:

300,000 - (140 x 60) = 291,600 mg O_2

This corresponds to a saturation of:

(291,600 ÷ 300,000) x 100 = 97.2%

1 hour after water has been shut off in a tank of 30,000 litres O_2 saturation is 97.2 % .

Tank volume = 5,000 l:

To find the total amount of O_2 in the tank:

5,000 l x 10 mg O_2/l = 50.000 mg O_2, that is, 50 g O_2

5,000 l contains 50,000 mg O_2

To find out how much O_2 remains in the tank one hour after the water has been shut off:

50,000 - (140 x 60) = 41,600 mg O_2

This corresponds to a saturation of:

(41,600 ÷ 50,000) x 100 = 83.2%

1 hour after water has been shut off in a tank of 5,000 litre O_2 saturation is 83.2 % .

This means that larger tanks show far better buffer capacity for O_2 when the water is stopped (and during insufficient water circulation).

Full saturation with oxygen, and all other gases, decreases as the water temperature increases. At the same time, O_2 consumption by the fish also increases due to increased metabolism. The water circulation in tanks should therefore be increased somewhat during the summer.

This can also be shown by a simple calculation:

Given 100 fish in a tank.

The water temperature is 12 ^{o}C and salinity is 34 ppm.

19.

Calculations:

Amount of O_2 consumed per minute:

O_2 consumption by the halibut increases due to increased water temperature. This has not been measured, but may be assumed to be 1.8 mg O_2/kg/min at 12 ^{o}C.

100 kg x 1.8 mg O_2/kg/min = 180 mg O_2/min

100 kg halibut consumes 180 mg O_2/min

Fully saturated water at 12 ^{o}C contains ca. 8.7 mg O_2/l (from table)

Water supply:

180 mg O_2/min ÷ 8.7 mg O_2/l = 21 l/min.

To replace the consumed O_2 at 12 ^{o}C a supply of 21 l/min of fully saturated water is needed. This is 7 l/min more than at 6 ^{o}C.

How does the halibut breathe when it lies completely still? It has a skin flap in both upper and lower jaws in the forward part of the mouth. These flaps move in phase with the outermost portion of the gill cover. The oral cavity and the gill cover act as a pump where the mouth flaps and the outermost portion of the gill cover function as one-way valves so that the water flow goes from the mouth and over the gills where the oxygen is taken up. An unstressed halibut pumps water over the gills about 20 times a minute (Personal observation). If there is a little bit of a current in the tank then the halibut can often be observed lying with the head into the current. The halibut thereby gets free help in pulling the relatively heavy water over the gills.

Gas supersaturation

Causes

Gas supersaturation in the water can often be a problem in halibut rearing since the selected technical solution is often a land-based system. In particular, attention should be paid to the danger of mixing air in the water system. This may happen by an accident of design of the water system, leaky hoses, false air in the pumps or the like.

During a loss of pressure the gas bubbles in the water develop a higher pressure than the surroundings and the gas diffuses gradually out from the gas bubble to the water at the same time as the bubble becomes smaller. This leads to supersaturation of, among other things, nitrogen and oxygen in the water.

An increase in the water temperature also leads to gas supersaturation. If the water is heated from 4 ^{o}C to 8 ^{o}C, a supersaturation of about 10% occurs. This may occur naturally in, for example, surface water in the fiords and inlets. If there is a real possibility of the temperature increasing in the water being used, then an aerator must be used. In addition, is should be ensured that there are no gas bubbles in the water in which the halibut swim.

Another cause of supersaturation in a facility is a change from high pressure to low pressure and the mixing of water of different temperatures.

Biological effect

Fish which live in supersaturated water may develop gas bladder disease. Greatly simplified, this may be compared to what happens immediately after one has opened a bottle of pop. Gas bubbles are formed in the fish in the same manner. These can be involuted and disappear if the fish is placed in water with no supersaturation or if it is placed in normally saturated water of a little greater depth.

The symptoms of gas supersaturation in halibut have not been researched, but the following have been identified from other types of fish (Berg, 1987):

- Protruding eyes
- Blisters underneath the outer skin.
- Small puncture bleedings.
- Abnormal/unusual behaviour.
- Bubbles in the vascular system.
- Bubbles in various tissues.

Of these symptoms, only protruding eyes is known as a problem

in halibut rearing, but the cause has not been identified as being gas supersaturation. Any gas supersaturation may, however, worsen this problem.

How much gas supersaturation the halibut can tolerate is unknown. For salmonids, the first problem occurs at around 5 % total gas supersaturation. This may be too high since the salmonids, in contrast to the halibut, live under conditions where supersaturation may occur periodically. It therefore appears that they have physiological mechanisms to reduce or prevent damaging effects from gas supersaturation.

Fish density

The halibut spends large portions of its time at the bottom, perhaps as much as 90 - 95 percent of the time for unstressed fish. A large bottom area is therefore preferable to a large volume in a technical solution. Furthermore, for that reason, fish density should be given as kg/m^2 and not as kg/m^3 as in the case of salmon.

Due to lack of research, it is uncertain what optimum fish density should be recommended per area unit (condition and size of halibut are contributing factors). Experiments have been carried out in Iceland with fish densities of 10, 20 and 30 kg/m^2. The results are still not complete (B. Bjørnsen,

pers. comm.). In the Islandlax facility, 3,000 small halibut are kept in 8-metre tanks at a density of up to 50 kg/m^2. Growth rate is said to be "satisfactory".

21.

For the time being, each fish farmer should use his/her own judgment, so that each halibut has a "reasonable amount of bottom area" available.

Observations regarding behaviour during rearing

In this section, some observations on the behaviour of reared halibut not dealt with anywhere else in this report have been compiled.

In some halibut of all sizes a behaviour can be observed which is normally interpreted as stress. The fish swims upward and part of the head comes out of the water. The halibut may butt against the tank wall without this stopping its behaviour.

Some halibut sometimes swim on their backs for a few seconds and scrape the back against the bottom of the tank. The reason may be that it is attempting to get rid of parasites, a behaviour that has also been observed in other fish.

Large halibut sometimes lie with their mouths wide open for up to 6 - 8 seconds. The reason is unknown.

Some halibut have a resting position on top of the substrata

where they lie in a slight curve so that both head and tail point slightly upward and only the middle portion of the belly touches the substrata. This observation has not been systematically studied, but some interpret it as a sign of stress (K. Senstad, pers. comm.).

If there is sand in the tank, then the halibut normally distribute themselves evenly over the whole bottom. In tanks with no sand, they are more apt to gather together and lie partly on top of each other (A. Kårdahl, pers. comm.).

Size differences in halibut often lead to a ranking being established. If they are lying still, dominant fish signal primarily by slapping with the forward portion of the left fin edge (personal observation). The movement of the fin edge increases in frequency with increasing aggression. This has not been systematically studied.

Where there are great size differences, the largest fish may eat the smaller individuals. It has been observed that large broodfish females have eaten smaller halibut. This may be a feeding reflex and not necessarily a result of aggression (see "Feeding", page 53).

FEED AND FEEDING

by

Ivar Rønnestad

Experience with feeding broodfish and consumer fish

Captured halibut kept in captivity can, with varying degrees of success, be trained to get used to artificial feed. The difficulty with this increases with the size at time of capture. It is sometimes necessary to feed whole fish to large halibut (>60 kg) as long as it is in captivity. This is not normally a problem as long as a vitamin mix with a Vitamin C supplement added is given to avoid vitamin deficiency. The mix is then added in the form of a capsule in the belly of the feed fish. On the Faeroe Islands, fish have been kept in captivity for more than three years without any vitamin supplements (I Fjallstein, pers. comm.). This required totally fresh fish and this type of feed will therefore be used only in exceptional cases. Since the halibut is an opportunist which eats what it comes across, feed fish can be whatever one has on hand, such as for example, herring, capelin, brisling, redfish, whiting, coalfish, etc., or squid.

When the greatest emphasis is on broodfish and particularly food fish, artificial feed must be used. So far, only soft feed has been used for fish larger than ca. 2 kg. The

reasons for this have been mainly practical, such as the size of the feed particles (dry feed can at present not be made larger than 13 mm in diameter), easy accessibility to fresh soft feed, flexibility in preparation, etc..

At the Aquaculture Station at Austevoll a soft pellet mix is used at present (see Table 1).

Table 1. Composition of soft feed used at the Aquaculture Station at Austevoll.

Shrimp shell	10.00 %
Herring meal	10.00 %
Salmon pellet meal 25%	34.50 %
Vitamin mix	1.00 %
Herring	44.50 %

This mixture is based on the halibut being a medium fat fish, somewhat fatter than cod, but significantly leaner than herring and capelin. During spawning season, the fat content of the fillet is 6 - 8 % (of dry weight), while it is considerably higher in red muscle, liver and fin bases (39 - 55 % of dry weight) (T. Haug, pers. comm.). If fillets with the same fat content as in wild caught halibut are desired, then the halibut feed will probably have to contain less fat than salmon feed (Ø. Lie, pers. comm.). The relatively large

input of shrimp shell is based on crustaceans having been found to constitute an important part of the diet of halibut smaller than 60 cm (McIntyre, 1952). In addition to providing some colour and more taste to the feed, the shrimp shell is a source of Ca2+. Squid and fish other than herring may also be used. A certain input of ensilage makes the feed more pliable.

23.

When looking at the composition of the actual feed, the percentage of dry weight is (Table 2):

Table 2. Theoretical composition of soft feed (percentage of dry weight).

	Spring herring	Small herring
Protein	55.3 %	56.0 %
Fat	12.3 %	10.8 %
Carbohydrates	20.6 %	20.8 %
Ash	10.0 %	10.1 %

The convertible energy/kg (MJ/kg) in the feed, based on digestibility models from salmon, are shown in Table 3. The percentage distribution of convertible energy is shown in brackets. As a comparison the recommended percentage distribution for salmon is also given.

Table 3. Convertible energy/kg in soft feed (MJ/kg) and as percent of total.

	Spring herring		Small herring		Salmon norms
Total	**8.4**		**8.1**		
Protein	4.9	(58.6%)	4.9	(60.8 %)	(30-50%)
Fat	2.2	(26.7 %)	1.9	(24.0 %)	(40 - 60 %)
Carbohydrates	1.2	(14.7 %)	1.2	(15.2 %)	(0 - 12 %)

As can be seen, the feed contains more energy from protein and carbohydrates and less from fat than does the salmon feed. It is uncertain whether the relatively high proportion of carbohydrates is beneficial. Salmon pellet meal, the largest contributor of carbohydrates, contains extruded wheat which normally increases the digestibility of carbohydrates for the fish. On the other hand, there are indications that the halibut digest carbohydrates much less readily than the salmonids (Grønseth and Myhre, 1988) so that the carbohydrates nevertheless cannot be utilized. In one experiment it was found that the digestibility coefficients for fat were 80 - 90 %, and for protein 80 - 85 %. Carbohydrate has a digestibility coefficient of only 6 - 7 %. The results also indicate that carbohydrate has a negative effect on the digestibility of protein and fat. The enzyme activity in the stomach and the intestines was also studied. Trypsin and protease activity was high in all intestinal samples but no amylase activity was found. Amylase activity is a

measure of the digestibility of carbohydrates. Based on this and concurrent results from other preliminary research (T. Hjertnes, pers. comm.), the feed mix will be evaluated.

The experiments carried out on Akvaforsk at Sunndalsøra used four different feed mixes of 8, 12, 16 and 20 % fat (% of wet weight), respectively, which all produced a growth of 0.3% per day (G.M. Berge, 1988). The fish used in the experiment was wild-caught halibut, which weighed ca. 1.3 kg at the start of the experiment. The water temperature was 7 ^{o}C. This may indicate that the amount of fat in the feed means relatively little to the growth of small halibut. An almost identical experiment was repeated on juveniles. In this experiment, also, there were no differences between high and low content of fat with regard to growth. It now only remains to be seen what significance the content of fat has on the fillets.

24.

At the other stations where halibut is kept, other types of feed than that used at the Aquaculture Station at Austevoll are used. At the University of Tromsø, a redfish-based soft pellet is used because redfish constitutes the main part of the diet of the local population of halibut. (T. Haug. pers. comm.). The (lack of) available redfish may limit this fish as a base for large-scale commercial pellet production in Southern Norway.

Until we know more about the composition of feed for halibut, every fish farmer should, first and foremost, concentrate on obtaining fresh, high-quality raw material for production of soft feed.

As previously mentioned, dry feed is used for all halibut under about 2 kg. The fish have grown equally as well on this as on soft feed, and the appetite has been satisfactory. The composition is shown below.

Table 4. Composition of dry feed used for halibut of less than 2 kg. at the Aquaculture Station at Austevoll.

Herring meal (LT-meal)	70.00 %
Capelin oil	17.50 %
Shrimp shell meal	8.00 %
Vitamin mix	2.00 %
Binder	2.50 %

Table 5. Chemical analysis of the feed.

Protein	52.0 %
Fat	27.5 %
Water	3.5 %
Ash	11.5 %
Carbohydrates	5.0%

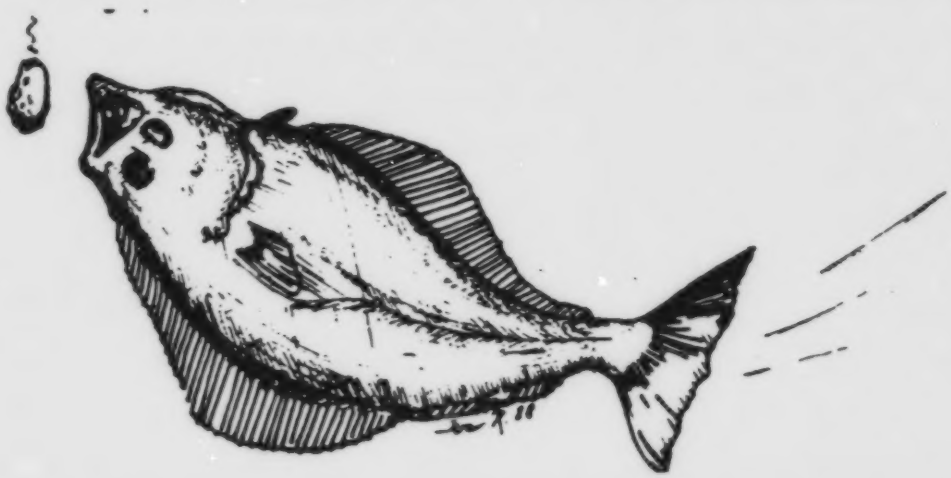
Feeding and feeding behaviour.

Large halibut are usually hand fed every second day. Experience has shown that large halibut prefer a large meal and then take one or two days to digest the food. We have elected to feed on Monday, Wednesday and Friday. The reason the halibut is hand-fed is that soft pellets are used and the fish takes a relatively long time to eat each pellet and, in addition to that, it provides better monitoring of the appetite and condition of the fish.

Halibut can feed both in the water and at the bottom. In both cases, an attack technique is used where the halibut approaches the feed straight ahead and then snatches it with a quick movement ("quick" is a relative concept, depending on the size of the halibut). What happens is that the halibut, with the aid of the gill cover creates a strong suction which pulls the feed into the mouth. It appears that the halibut cannot notice the food if it is thrown too close to it, particularly if the food is on the halibut's right side (I. Karlsen, pers. comm.). If the halibut does notice the food, then it often starts over again and executes a new attack. Some halibut are more mobile than others and can "turn on a dime" and do not need this elaborate attack tactic.

Live feed can also be used for halibut even though the results

vary and the ethics are dubious. Brisling and rainbow trout have been used with good results (the price of rainbow trout dictates that the question of using rainbow trout as feed fish on a large scale will not arise). On the other hand, coalfish



is often too quick for the halibut, at least in small tanks. If the feed fish is not eaten at once, the halibut become used to it and only show interest if the feed fish is damaged or the like.

In some cases, the feed, pellets or fish must be presented to the halibut by being dragged through the water so that an eating response is provoked. It appears that the halibut snaps at everything of a more or less appropriate size, is in motion and preferably has a light colour (such as, for example, an O_2 measuring probe or the belly side of other halibut in motion). This indicates that vision is important during feed uptake.

To avoid deterioration of the water quality, feed not eaten when feeding is completed should be removed from the tank.

The appetite of the halibut varies with the seasons. Halibut of all sizes eat less during the winter. It is not clear whether this is because of the temperature, the light or, possibly, other factors. Broodfish, particularly female fish, often do not eat at all during the spawning period. This may be because the fish is continuously stressed in connection with stroking, but also the halibut is not normally caught on hook-and-line during this period.

In some cases, the fish must be force-fed. The following method can then be used: Water is added to some of the previously mentioned mixture (ground up fish or the like), so that the mix becomes fluid. A large syringe mounted on a rubber hose (1 - 3 cm in diameter) is filled with the mix. The consistency of the feed mix must be such that it can be relatively easily forced out of the syringe. The rubber hose is inserted into the throat of the halibut, which is monitored on a canvas sail while feeding is in progress.

If the fish is going to be subjected to stress, for example, in connection with transfer, then it must not be fed two days prior to this or it will regurgitate the food.

GROWTH

by

Ivar Rønnestad

As mentioned in the chapter "Natural Biology of the Halibut", the halibut grows very slowly in the wild. This applies particularly to the first years of life. In the wild, the male halibut will not exceed 10 kg in weight until after age 8 - 9, while the females reach this weight at the age of 6 - 7.

It is therefore very important the determine whether the halibut has a growth potential in a rearing situation which would make it economically viable to aim for halibut rearing as a future industry. Research must still be carried out to determine the optimum size for slaughtering with regard to economics and growth.

Few studies have been carried out on halibut growth during rearing. Rabben and Huse (1986), found that halibut caught in the wild grew an average of 1.7 kg to 5.8 (0.29 % per day) during 14 months of rearing. Some of the fish reached a weight of about 7 kg at the end of the experiment. The experiments lasted from June 1985 to August 1986 and therefore included the best growth periods in both years. It has been found that

small halibut caught in the wild can grow as much in one year in captivity as in two years in nature (Haug et al, 1988).

We now have a certain data base for a growth curve extending over the three first years of life (Figure 5).

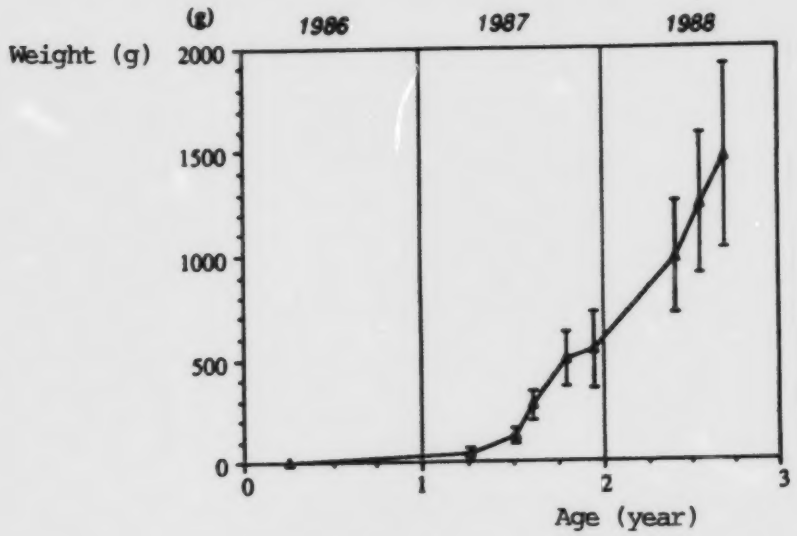


Figure 5. Weight (in grammes) recorded for 1986 juveniles. The data are up-to-date as of November 1988.
(Rønnestad and Kårdal, 1988).

28.

This curve is based on measurements carried out on the 1986 generation, consisting of 67 remaining halibut. This may indicate that the increase in growth of the wild-caught halibut which was observed in Rabben's and Huse's experiments

represents a somewhat optimistic growth potential among domesticated halibut. On the other hand, it will probably be possible to increase the growth of consumer fish (food fish) by optimizing factors such as temperature (see below), fish density, feed, tank size, etc.

The relatively large standard deviation of the measurements indicates a large spread in the growth. The smallest halibut is only 430 g while the largest weights 3050 g (see Table 6). When rearing halibut, individual differences in growth will be an important basis for selection.

It is worth noting that the larger halibut (of two) from 1985 was only 2622 g which is 430 g less than the largest in the 1986 batch. There may be many reasons for this, such as stress, individual variations in growth, and so on.

Table 6. Growth (in grammes) recorded for halibut hatched at Aquaculture Station at Austevoll. The data are up-to-date to November 1988 (Rønnestad and Kårdal, 1988).

Generation	Weight (g)	Max (g)	Min (g).
1988 (207 fish)	13	25	1
1987 (21 fish)	238	415	20
1986 (67 fish)	1440	3050	470
1985 (2 fish)	2282	2620	2229

Effect of water temperature

Among all species of fish there is a clear correlation between oxidation and temperature. This leads to increased water temperature providing increased growth if not limited by other factors (such as feed uptake, content of O_2 in the water, etc.). We know little about the effect of temperature on growth in halibut and so far only one experiment has been carried out at the Aquaculture Station at Austevoll where these effects are being researched (Hjertnes, 1987). In this experiment, two different water temperature were used, 8 ^{o}C and 14 ^{o}C. The duration of the experiments was 6.5 months. The fish used was one (1) year old at the start of the experiment.

The results showed that at a water temperature of 8 ^{o}C an individual increase in growth of 178 g was measured (See Figure 6). This fish increased in weight from 35 g to 213 g (0.93 % per day). The other group which was kept in water having a temperature of 14 ^{o}C increased in weight from 40 g to 348 g, that is, an added growth of 308 g (1.11 % per day).

29.

This clearly indicates a positive effect of increased water temperatures on the growth of halibut of this size. It is still uncertain at which temperature growth could be

optimized. Experiments show that rapid temperature increases from 10 ^{o}C to 13 - 14 ^{o}C lead to reduced growth (I. Holmefjord et al, 1986).

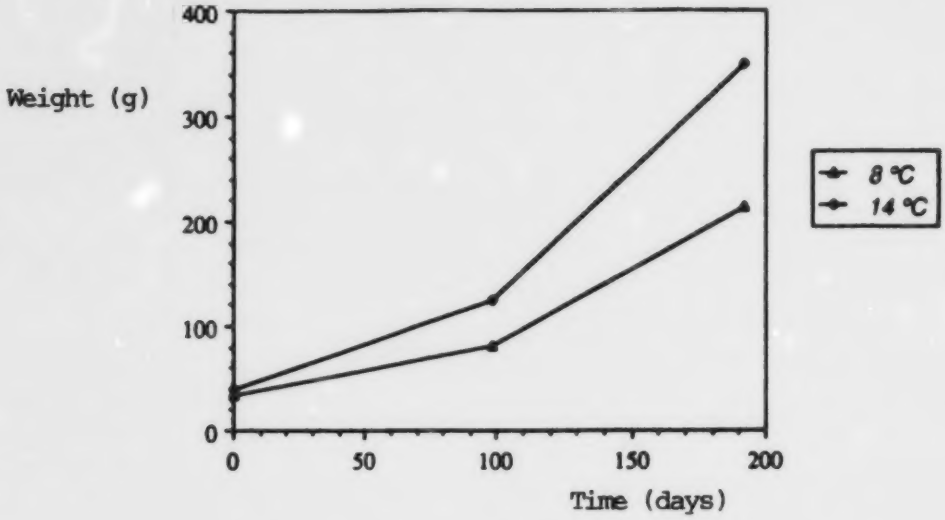


Figure 6. Growth of halibut at 8 and 14 ^{o}C water temperature. (Hjertnes, 1987)

HYGIENE

by

Emmy Egedius

Hygiene in fish farming is very important to avoid diseases and to increase comfort. This paper describes the minimum measures that should be implemented at all land-based halibut rearing facilities which are not fenced in and are on foundations which are difficult to disinfect. In these cases it is impossible to implement some of the normal hygienic measures such as, for example, foot baths (a mat dipped in disinfectant).

This may/should be implemented:

Each tank should have its own cleaning equipment (broom and dip net) which is disinfected after use. Alternatively, such equipment may be used in all tanks, but then there must be enough such equipment so that any items used can be left in liquid disinfectant for at least 15 minutes after each use. It is simplest, in such cases, to have two containers of liquid disinfectant with all the tools and equipment in one container and place them in the second container after use.

The tanks should be cleaned and disinfected after use.

Personnel working at the facility must have their own work clothing (coveralls and boots) which is used only at the site.

Visits should be limited as far as possible.

A sign should be displayed warning visitors that particularly WATER, FISH and TAPS, must not be touched.

Signs should be posted warning of no entry to the facility by unauthorized personnel.

Protective equipment (masks, eye protection, gloves and protective clothing) must be available at the facility and used when necessary.

Disinfection

To disinfect equipment, a 2 % J2 solution should be used. This solution is brown in colour. It must be changed regularly, at least every 14 days. When the colour turns light brown it is an indication that the solution is no longer active and must be changed.

To disinfect larger equipment and items one does not want to have stained brown, chlorine (hypochloride, chlorine, chloramine, chloramidol), may be used.

We overdose when we use chlorine; 0.2 g active chlorine per

litre is a normal solution for use. (Hypochlorite: 20 ml per 10 litres of water, chlorine 50 ml per 10 litres of water, chloramine and chloramidol: 20 g in 10 litres of water). Remember that chlorine is a potent fish poison so that the equipment must be very well rinsed after use. Formalin must not be used for disinfection, since it is hazardous to health. When using formalin in large quantities, protective equipment must be worn.

31.

Washing

The veterinary authorities recommend caustic soda (NaOH) for washing tanks. Solution to use: 2 promille, that is, 20 g to 10 litres of water, may be used in a high-pressure wash.

DISEASES

by

Odd Magne Rødseth

and

Ivar Rønnestad

Experience gained from all forms of domestication and rearing of wild animals has shown that the disease frequency increases drastically. Our own farming of salmon and rainbow trout and the rearing of various marine species from abroad have shown that disease is probably the one single factor which imposes the greatest limitations on the development and growth of the industry.

Even though disease in halibut has been little researched, various infectious diseases and enviromentally related ailments will probably represent the greatest loss factor in future intensive rearing of halibut. It is therefore important already now to start mapping out possible disease-related problems and the possibilities of effective prevention.

In this chapter the most important diseases which have already been identified (marked Identified) or which may represent a problem in halibut rearing (marked Potential) will be reviewed.

Viruses

More than 50 different types of viruses have been described as being capable of causing diseases among fish. Of these, about 20 have been identified among marine fish species. Table 7 presents an overview of the types of viruses which may create problems when rearing halibut in Norway.

Table 7. Potential virus diseases when rearing halibut.

TYPE OF VIRUS	SPECIES	SPREAD[1]
LYMPHOCYSTIS VIRUS	CA 130 SPECIES	I.O.V.M.B.F. WORLDWIDE
VIRAL ERYTHROCYSTIC NECROSIS VIRUS (VEN)	CA. 130 SPECIES	E.O.V.M.B.F. WORLDWIDE
INFECTIOUS PANCREAS NECROSIS VIRUS (IPNV)	CA. 40 SPECIES	I.O.V.M.B.F. WORLDWIDE

1

E: Identified by electron microscopy

I: Identified by isolating the virus

V: Found in wild fish

O: Found in farmed fish

M: Sea water

B: Brackish water

F: Fresh water

It is very natural to believe that Lymphocystic viruses, which must be considered as "universal pathogens", will be the first viral disease to be encountered in halibut rearing.

33.

Lymphocystis (Potential)

The lymphocystic viruses attack primarily the skin and connective tissues of the fish. Infected cells swell up noticeably and can be observed as grey/white nodules under the fins and/or the skin of the fish. The nodules may vary in size from two to ten millimetres, and can often be found in clusters. The characteristic signs of the disease makes it relatively easy to diagnose the problem based solely on macro-scopic observation. When the nodules break open or fall off, large amounts of virus particles are released which may cause new infections. This is considered the most important route of infection. There are no indications that lymphocystic virus can be transmitted through eggs (vertical infection).

The disease is common among the wild fish population. In certain areas, for example, on the Skagerak, Kattegat and the Dogger Banks, infection frequencies of 20 - 30 % in individual species have been recorded (particularly in flounders). There is therefore a distinct probability that this disease will be introduced when rearing halibut.

Lymphocystis rarely results in high mortality, but the nodule formation on skin and fins makes the fish unappetizing and therefore unsuitable for sale.

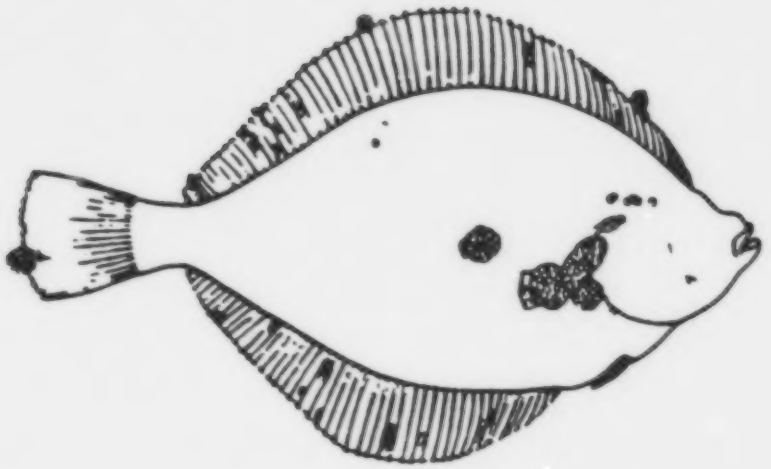


Figure 7. Lymphocystis in flounders. Note the nodules which are clustered on fins and in the areas around the gill covers.

Many viral infections cause chronic diseases which are not related to massive mortality. However, it has been demonstrated several times that virus-infected fish have increased susceptibility to other diseases, such as, for example, vibriosis.

Bacteria

Regardless of the fish species being raised in sea or brackish water, vibriosis will often be the first disease to be introduced into the facility. Up to 1980, vibriosis was the greatest loss-causing disease in salmon farming, and this

bacterial disease has now been the cause of a number of outbreaks among marine fish species in fish-farming (cod, turbot and halibut). Vibriosis is therefore the disease which can with certainty be said to represent a problem in all forms of intensive farming of marine fish.

Egusa (1980) presented an historical review of the development of diseases in fish farming of Yellowtail (Table 8). This illustrates how various diseases are accumulated at the introduction of new fish species in fish farming. A similar development has been experienced by us in the salmon farming in Norway. It is likely that the rearing of halibut will follow a similar pattern.

Table 8. Microbial diseases in rearing of Yellowtail (Egusa, 1980.

DISEASE	1960 1965 1970 1975 1980	LOSS (£. 1978)
VIBRIOSIS	XXXXXXXXXXXXXXXXXX	2.2 MILL.
NOCARDIOSIS	XXXXXXXXXXXXXX	0.5 MILL.
ICHTHYOPHONUS-INFECTION	XXXXXXXXXXXXXX	0.5 MILL.
PSEUDO-TUBERCULOSIS	XXXXXXXXXXXX	12.5 MILL
STREPTOCOCCUS-INFECTION	XXXXXXX	30 MILL.
LYMPHOCYSTIS	XXXXXX	0.5 MILL.

Sore formation/fin rot (identified).

In connection with handling of fish (catching, stroking, transporting), the fish often suffers mechanical damages to the skin/fins. Injury may also occur as a result of aggression. Once the fish's outer barrier is broken (slime and skin layers) then the sore will quickly be colonized by bacteria from the environment. The result is often a further expansion of the skin sores and baring of the fin rays, which, in the worst scenario, would lead to loss of the whole fin. Local bacterial infections on the fins may also be passed on to the skin around the base of the fin, and complicate the situation further.

No specific types of bacteria can be said to be the cause of this type of ailment. A mixed bacterial flora can often be found in connection with such sore formation, but Flexibacteria, in particular, appear to dominate the bacterial flora in sore formations on halibut.

Vibriosis (Identified).

The bacterium which causes vibriosis, **Vibrio angullarum**, is a natural part of the microbial flora in the marine environment. This means that the infection can be found everywhere in brackish and salt water, and that the fish are continually

exposed to this disease-causing bacterium. The disease is often unleashed by the fish being subjected to various forms of stress. The fish is particularly susceptible to vibriosis during the juvenile stage, and then mainly at temperatures above 10 ^{o}C. At the Aquaculture Section of the Oceanographic Research Institute, we have only found vibriosis on halibut juvenile younger than 6 months, calculated from metamorphosis. On other marine species such as cod, turbot and sole, we have found vibriosis among fish of all sizes and ages. 35.

The most typical external sign of the disease is bleeding of the skin and musculature, and ecchymosis at the fin bases. In many cases, protruding eyes may also be observed. In cases of acute outbreak of the disease, high mortality can be experienced without any particular symptoms.

Cold water vibriosis (Hitra disease) (Potential).

Cold water vibriosis (CV*) is a disease which has caused the fish-farming industry the greatest losses in the last 6 - 8 years. Recently CV was found on reared cod in Finnmark. Little is known of how susceptible various species of marine fish are to this disease, but it cannot be excluded that even halibut can be infected by CV.

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* KV = Norwegian abbreviation. Tr.

Fish tuberculosis (Identified in wild fish).

Mycobacterial infection, or fish tuberculosis, has been described in more than 150 species of fish in both fresh and salt water. In Norway, the disease has been recorded in broodfish for salmon, wild fish in Glomma, wild fish in the North Sea (particularly in mackerel) and in aquarium fish. The development of the disease may be slow (chronic) and it may take up to 4 years before infected fish develop visible signs of the disease. The picture of the disease may vary, but loss of appetite, lethargy and loss of weight can often be observed. Autopsies have found nodules and tumours in organs such as the kidney, liver and spleen to be the most pronounced. There is no effective treatment for mycobacterial infections.

There are a number of other infections which produce pictures of diseases similar to those found in mycobacterial infections. **Pasturella piscida** presents a similar picture and the disease is also known as pseudotuberculosis. The disease may occur as acute or chronic and occur mainly at high water temperatures (>18 ^{o}C) and in poor quality of water. External signs are often abnormally dark pigmentation, bleeding in the eye region and at the base of the fins. Autopsies reveal the same infections as for mycobacterial infection.

Fungi (Potential)

In the marine environment there is one particular type of fungus disease that is relatively common in wild fish populations. This is the so-called **tumult disease**, which is caused by the fungus **Ichthyophonus hoferi**. In contrast to most of the other fungi ailments, this fungus causes an internal, so-called systemic, infection. This means that the fungus spreads through the blood and attacks the fish's internal organs. Organs which have large blood flow (kidney, heart, liver and spleen) are particularly vulnarable. The extent of tissue damage and the symptoms which can be observed vary sharply between the different species of fish. In many cases, the brain of the fish and the central nervous system are attacked. The fish will behave irrationally and with uncontrollable swimming motions and muscle cramps; hence the name **tumult disease.**

36.

The other common symptoms of tumult disease are apathy, dark pigmentation and protruding eyes. Autopsies have found white nodules in the heart, liver, spleen and kidney. In herring, **Ichthyophonus hoferi** infections present a characteristic picture of the disease. The fungus invades the musculature and inner layer of skin (dermis). The nodule formations and damage to this tissue give the infected fish a rough surface ("sandpaper effect").

The fish probably becomes infected by way of the stomach/intestinal tract. The fungus spores are released under the influence of the

digestive juices. They can penetrate the intestinal wall and be transported by the blood to the various organs. Infected fish may release spores through defecation which, in the next stage, may cause new infections. The spores are also very resistant and may survive for at least 6 months in sea water without losing their pathogenicity (disease producing ability).

Ichthyophonus hoferi can be found world-wide. A number of outbreaks of the disease in wild fish, often with great mortality, have been reported. Outbreaks of the disease have reduced the natural population of herring, mackerel, haddock and flounder by up to 50 % in certain areas, particularly on the East Coast of the U.S.A. (the Gulf of St. Lawrence) and along the coast of Scotland and the Orkney Islands.

There are two aspects, in particular, which makes tumult disease a potential risk in future halibut rearing.

1. The disease is relatively common in the North Sea. A reservoir of infection already exists in wild fish. This can easily be transferred to fish farm populations.

2. Marine copepods can probably function as intermediate hosts for **I. hoferi**. Copepods are, as is known, natural prey for halibut larvae.

There is no effective method of treatment for this fungus disease.

The spread of infection can be reduced by systematic collection and destruction of the dead/dying fish. In facilities where wet feed of marine origin is used (particularly herring, brisling, mackerel), a certain risk of introducing the disease into the facility must be expected. The danger of infection will be reduced when using frozen or ensilaged fish. The fungus spores are very resistant, however, and could survive even in frozen or acid-treated state.

Parasites

About 10,000 different parasites which live on or in fish have been described. Parasites can be divided into two categories:

1. Endoparasites: Parasites which live internally in the fish's organs or abdominal cavity.

2. Ecto-parasites: Parasites which live on the fish's external layer of skin, that is, skin or gills.

Up to now, parasites have been the greatest problem in the rearing of halibut. This applies particularly to the halibut fluke, but halibut lice and **Trichodina** have also been reported.

37.

Parasites are widespread in wild fish populations. In natural populations, there often exists a equilibrium between parasite and host, so that the parasite does not cause the host serious

damage. In an intensive rearing situation, this equilibrium is often displaced in favour of the parasite. High density of fish often makes it easy for the parasite to find a host. A stressed fish cannot offer sufficient "resistance" to an attack of parasites which then, directly or indirectly, results in mortality.

Examination of parasites on fish

A number of parasites are so large that they can be observed with the naked eye. But the majority of fish parasites require special equipment, such as a magnifying glass or a microscope to be found.

Among the general clinical signs which can be observed in attacks by ecto-parasites are:

- The fish becomes restless, with irrational swimming motions (turning, "flashing").
- Ecto-parasites often provoke increased slime production in the host. This can be observed as a grey/bluish coating on the surface of the fish (as is caused by, for example, Trichodina).
- When the gills are attacked, the breathing frequency will become abnormally high. The fish can often be found near the water intake of the tank.

Examinations of ecto-parasites should preferably be carried out on live or recently-dead fish. Quite a number of parasites will die or leave the host immediately after slaughter.

Slaughter should be carried out by striking the fish on the head or by bringing a scalpel down between the brain and the spinal cord. The use of anaesthetics often will immobilize or kill the parasite.

Small fish to be examined should be moved from the tank into a bucket of water by use of a dip net. Avoid touching the fish with your hands. Use a pair of tweezers and transfer the fish from the dip net to a glass plate or a slide (do not use paper which absorbs water). After slaughtering, scrape the body sides and fins using the scalpel and transfer the slime to a clean slide. A drop of water is then added to the preparation before the cover slide is placed on top. The preparation is immediately examined under a microscope (preferably a microscope with phase contrast).

The same procedure is used when examining the gills. It is often an advantage to cut away some of the gill arch and place it on the slide before scraping off the slime.

It is also important that some of the gill be formalin-fixed immediately after slaughtering. This will be of great significance for later histological examinations.

38.

Ecto-parasites

The Halibut fluke (Identified).

Until now, the halibut fluke (**Entobdella hippoglossi**) has represented the greatest disease problem in the rearing of halibut. It settles all over the body of the halibut, even on the underside, but appears to prefer the head section (see Figure 7). It is most commonly found on large halibut, but can be found even on small ones. It is white in colour and has an oval shape and can become up to 1.7 cm long. The inset drawing shows the details.

In nature, the halibut fluke will not normally present a problem before the halibut visit the spawning grounds. Intensive rearing is ideal for the blooming of the halibut fluke. Attacks cause the fish to lose appetite and, in the end, it may die.

Halibut lice (Identified).

Halibut lice (Lepeophtheirus hippoglossi) are similar to salmon lice (See Figure 8). They have been identified on wild fish and broodfish.

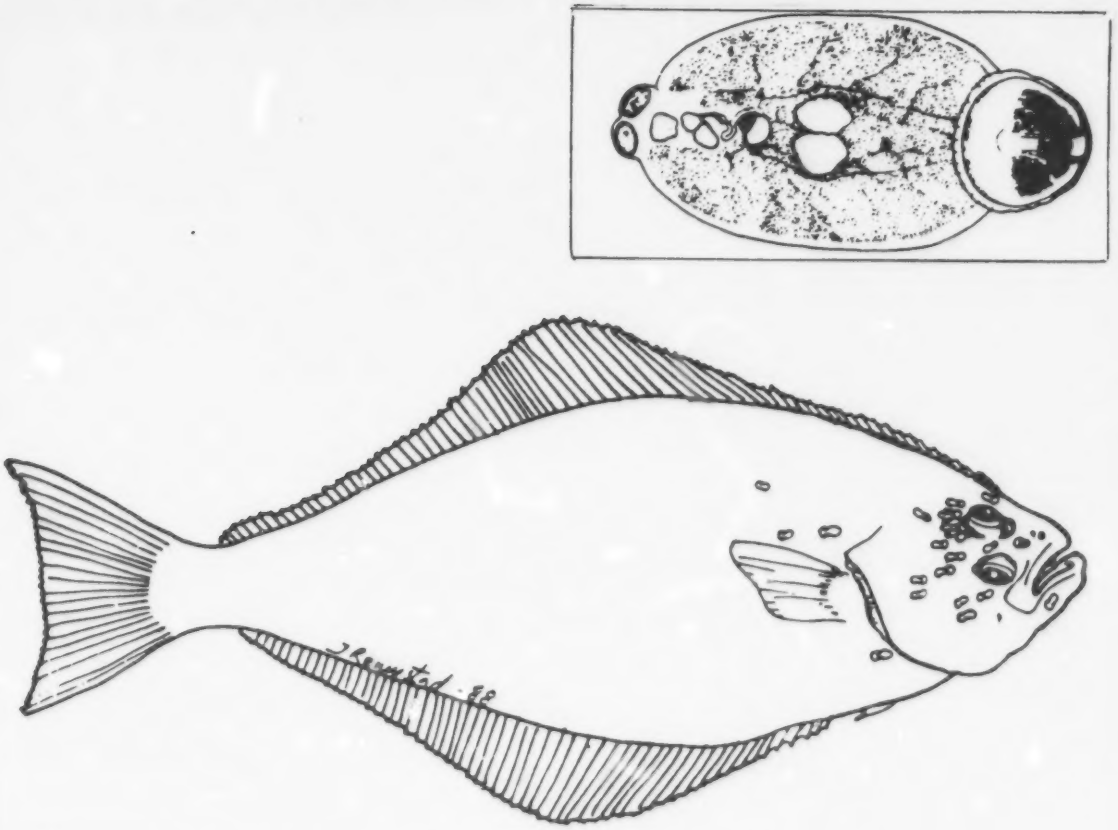


Figure 7. Sketch of a halibut infected with halibut flukes (Entobdella hippoglossis). An enlargement of the fluke is inset. (From Ny Fisk, 1988).

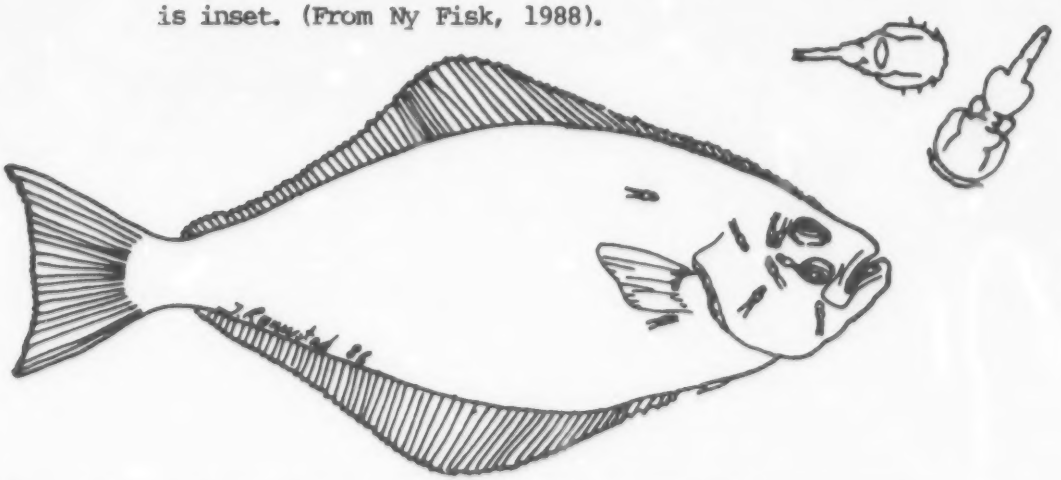


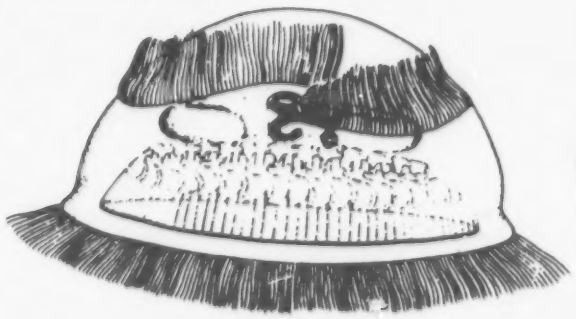
Figure 8. Halibut lice (Lepeophtheirus hippoglossi).

The first serious infection of halibut lice was reported in July 1988 at a facility in the Faeroe Islands (I. Fjallstein, pers. comm.). Three halibut were heavily infested in the course of a short period. Treatment with formalin was started but this did not prevent the fish from developing large sores concentrated around the gill covers. The injured halibut were treated with antobiotics with good results.

Trichodina spp. (Identified).

This group of parasites is relatively common among most of the marine species of interest to fish farming. The first sign of a Trichodina infection among halibut is a grey/bluish coating on the skin. If treatment is not started at an early stage the parasite may cause mortality among juveniles, food fish and broodfish. It is therefore important to routinely examine for parasites (as well as a daily inspection). The parasite can easily be found when examining skin and gill scrapings under the microscope. **Trichodina spp** can become up to 0.1 mm in diameter, circular and with a characteristic "skeleton" (Figure 9).

Figure 9. Trichodina spp.



The parasite fastens onto the host's epithelial cells (skin cells) with a suction cup. Formalin treatment is usually effective.

Ichthyobodo necator (Costiasis) (Potential).

Ichthyobodo necator, better known by the name Costia, was originally described as a skin and gill parasite among fresh water fish. However, in recent years, the parasite has been found among a number of marine fish species. Routine examinations at the Oceanographic Research Institute (Havforskningsinstituttet) have discovered Costiasis on both turbot and cod. We have yet to demonstrate Costiasis among halibut but it is natural to believe that even the halibut can function as host to this parasite.

I. **necator** occurs in several morphological forms (Figure 10).

1. **Attached form.** The parasite is attached to epidermal cells on the skin/gills. May be observed in histological preparations as a pear-shaped structure, size about 8 -12 um.

2. **Free-swimming form.** Oval or bean-shaped with two flagellates. May be observed by microscopy of gill skin scraping. This stage is probably responsible for transfer of the infection (hortizontal infection).

3. **Cystic form.** Under unfavourable (to the parasite) environmental

conditions it can form cysts in the skin/gill section. This stage is somewhat disputed, but persistent reports conclude that a cystic stage does exist.

Figure 10. Ichthyobodo necator (Costiasis). Attached form (A) and free-swimming form (B).

Water temperatures of between 10 and 20 ^{o}C favour rapid reproduction of the parasite. Large numbers of free-swimming parasites and loosened skin and gill epithelium can then be observed. Under such conditions the gill tissue will be rapidly damaged and high acute mortality may be experienced. At water temperatures below 8 ^{o}C the activity is considerably reduced and the parasite will form cysts. This form is inactive and will not cause any damage.

The first sign of costiasis is usually that the fish becomes restless and rubs against the tank wall. After a while, the fish develops a

grey/blue sheen due, among other things, to increased production of slime. When the gill tissue is attacked, the fish will place itself high in the tank, often near the water out-let, and its breathing frequency is sharply increased. Repeated formalin treatments are often necessary to cure the fish.

41.

Caligus curtus.

Caligus curtus is a copepod, which is a group within the crustaceans. So far, it has been observed at one facility (K. Hamre, pers. comm.). The parasite will attach itself to the juvenile. When infected, juveniles exhibit a peculiar behaviour where they do "somersaults" in the water, probably to get rid of the parasite.

Other

Fishermen in Saltstraumen have observed that shells can attach themselves to and grow on the skin of halibut. This has not been observed in captivity at Austevoll and does not represent any problem.

Endo-parasites (Identified).

Tapeworm (Cestoda) and roundworm (Nematoda) are endo-parasites which will probably be introduced through intensive rearing of halibut. Histological examinations at Havforsknings-instituttet (the Oceanographic Research Institute) have discovered tapeworms

in the intestines of newly-metamorphosed halibut juveniles. At the autopsy of a recently dead juvenile from the Aquaculture Station at Austevoll, roundworm (Hystiothylasium spp) was also identified (B.Berland, pers. comm.). The path of infection is probably through copepods which often function as intermediate hosts in the life cycle of the tapeworm.

Most of the tapeworms and roundworms which are known to be parasites on fish apparently have no seriously harmful effects on the host organism. In those cases where the parasite occurs in large quantities, it can probably create serious problems in the form of decreased comfort and growth. Questions can also be raised about the harmful effects on newly-metamorphosed halibut juveniles.

Medicamental treatment.

At present, practical measures against diseases in aquaculture are, to a far too great degree, limited to the use of antibiotics, chemotherapeutics and antiparasitic agents. The elimination of infectious substances through the use of these agents may be effective. But, regardless of efficacy, this form of treatment must be considered a temporary fix. The occurrence of disease is, in most cases, the result of a number of interactions between the host, the environment and the disease-producing organism (pathogen). In order to prevent

diseases in the long term, an evironment must be created which both prioritizes the health and comfort of the fish, and minimizes the occurrence of disease-producing micro-organisms in the environment. If that is succesful, then, at the same time, an environment is created which eliminates most of the pathogenic micro-organisms.

Intensive rearing of halibut will probably also depend on the use of medications. It is therefore important that fish-farmers who employ the various treatments be informed about the use of the different medications utilized in aquaculture. It is a recognized fact that many of the substances used today may cause lasting harm to people and fish when improperly used (See also "Hygiene"). In the following, a brief overview of the most frequently employed treatment methods will be presented.

42.

Treatment against bacterial diseases.

Systemic bacterial infections (infections where bacteria attack the fish's internal organs, spread by way of the blood) are most often treated with antibiotics added to the feed. The type of bacteria which caused the infection determines the type of antibiotics to be used. To be able to implement effective treatment it is therefore important to isolate the disease-producing bacterium and examine its sensitivity to the

appropriate antibiotics. Table 9 presents a summary of the antibiotics and chemotherapeutics most commonly used for farmed fish.

Table 9.

Summary of the antibiotics and chemotherapeutics most commonly used for farmed fish. Those used, so far, for halibut at the Aquaculture Station at Austevoll are marked with **.

Antibiotics/ Chemotherapeutics	Dosage mg/kg fish/day	Number of treatment days
Oxytetracycline **	75 - 100	8 - 10
Nitrofurazolidine	75	10
Erythromycin	100	21
Tribissen	75 - 100	10
Oxolinic acid	10	10
Flumequin **	12	6

A practical tip to those who use dry feed and mix the medication at the facility: After having accurately calculated the dosage, the medication is mixed with capelin oil. This mixture is sprayed on the dry feed. If a large

amount of medicated feed is needed, then use a cement mixer (washed and disinfected) for "coating" the medication. Capelin oil will act as a taste attractant (in addition to sick fish having a decreased appetite, many medications are unattractive) and it also reduces the possibility of the medication being washed out during the treatment.

Even though the use of medicated feed is easy and less stress-inducing for the fish, there are many disadvantages in this method of treatment.

- Sick fish often have reduced appetite. This results in the fish which most needs the treatment obtaining the least medication.
- At low water temperatures, feed intake is sharply reduced.
- Many of the medications used today are poorly absorbed through the intestines.
- Wastage of feed results in remnants of medications becoming a problem.
- Correct dosage is dependent on the fish farmer, at all times, having an overview of the total biomass to be treated.

At land-based fish farming facilities, bathing fish in medicinal solutions may be an appropriate treatment method.

This ensures that all fish obtain the same amount of antibiotics, regardless of whether the fish eats or not. Some medications can be well absorbed from saltwater. Absorption takes place either directly over the gills and the lateral lines, or by the fish drinking water, (fish in sea water drink a lot of water to avoid dehydration) and the antibiotics are absorbed through the intestines. Excess medication can here be collected and destroyed before possibly being released into the sea. 43.

When treating valuable broodfish, intraperitonal injections (antibiotics are injected directly into the abdominal cavity) have been found to be successful.

Treatment against ecto-parasites

A number of chemicals have been described which can be used in the treatment of ecto-parasites among fish (Herwig, 1979). Here, we will review two of the most common treatment procedures against ecto-parasites on halibut; formalin treatment and fresh water treatment.

Formalin treatment.

Formalin is primarily used against attacks by the parasites **Trichodina spp.**, the halibut fluke and **Ichthyobodo necator** (Costiasis).

Formalin acts to reduce and coagulate proteins. It kills parasites on fish skin and gills. The therapeutic effect is based on different toxin tolerances between the fish and the parasite. The therapeutic index (the relationship between lethal concentration to the parasite and the host) is about 2. This is low compared with other medications used in aquaculture. The danger of direct mortality or irreversible damage to the fish must therefore be regarded as relatively high. In salmon rearing there are numerous examples where sloppiness in dosing, or treatment under unfavourable environmental conditions resulted in mortality of the fish.

Factors which will increase the toxic effect on the host, that is, the halibut:

- If the fish's gills are already deteriorated due, for example, to bacterial gill infection.
- High water temperatures

- Formalin can react with organic substances in the water, for example, feed remnants and feces, to create toxic substances.

- Formalin stored at low temperatures or exposed to light results in the formation of white precipitates (paraformaldehyde). This substance is very toxic to the fish, and such formalin must not be used in treatment.

Formalin is very allergenic and also potentially carcinogenic. Those working with the substance must therefore use special protective clothing (mask, eye protection, gloves, coveralls). In addition, dosing syringes should be used to avoid evaporation of the formalin. This particularly applies when using formalin indoors (see also "Hygiene in fish farming").

Treatment procedure

Recommended dosages:

- Preventive treatment 1 : 6,000 (formalin:water)
- Therapeutic treatment 1 : 4,000

Practical implementation of therapeutic treatment as it is carried out on halibut. (Preventive treatment is not yet common):

- The fish is starved for 24 hours before treatment.

- The tanks are thoroughly cleaned, and particulates in the water must be avoided.

- The exact volume of water in the tank is calculated (after the water level has been lowered). The number of litres to be added to the individual tanks is calculated.

- Oxygenation equipment is made ready (bottle, manometer, grating and oxygen gauge).

- Formalin is diluted ahead of time in a bucket/tank. NOTE! Do not add pure formalin directly to the treatment tank.

- The water level is lowered (10 - 20 cm).

- Formalin is added gradually as water is added.

- Shut off water supply when all the formalin has been added and the water level has reached the calculated treatment volume.

- Oxygen is added. Oxygen saturation should be about 80 - 90 % during the treatment. Use an oxygen gauge.

- Treatment period: 20 - 30 minutes. Then turn on the water supply so that the formalin is diluted gradually.

The treatment should be carried first out on a small number of fish to check that the arrangement functions properly. Then wait about 12 - 24 hours before beginning the main treatment. During this period, the fish must be observed frequently. The halibut should also be monitored continuously during the whole treatment. In case of abnormal behaviour or mortality, the treatment must cease immediately by fully opening the water supply. The dilution can be increased by simultaneously draining water from the tank.

The fish must not be scrubbed or the like during the treatment since the parasites will die and fall off after a period of time.

Procedure with fresh water treatment.

Treatment with fresh water is employed on some of the other flatfish and is a gentler form of treatment of the fish. Despite the halibut being a saltwater fish, it will probably not experience great problems with the water and the salt balance for the short time that the treatment will last.

The common treatment method when using fresh water is based on isolating the fish in a smaller tank which floats in the tank in which the fish is normally kept. Instead of a tank, a small rubber float or the like may be used. The tank, or the

float, is filled with fresh or brackish water and the halibut is placed in it. At the conclusion of the treatment it is important that the treatment water not be mixed in with the remaining volume of water.

If 100 % fresh water is used, then a treatment time of 40 minutes has been found sufficient to remove all halibut flukes (W. O'Connell and T. Haug, pers. comm.). When a certain amount of salt water is mixed in, a somewhat longer period is needed to eliminate the flukes. The halibut will be quite drowsy from this treatment and will be easy to handle afterwards.

45.

Supplement when treating halibut fluke (Entobdella hippoglossi).
A better knowledge of the generation period of the halibut fluke has made it possible to combat it effectively. The treatment is as follows (William O'Connell, pers. comm.):

- Carefully remove some flukes and set them in cultures some time before beginning the treatment; they may live in a normal petri dish with salt water for several weeks. Use 100 ml salt water/individual.

- In this culture the generation period is studied. This period is calculated from the egg to adult egg-producing individual. The generation period is dependent on

environmental conditions such as, for example, temperature. The halibut fluke starts producing eggs when it is about 4 mm. It then produces one egg per day. At a size of 5 mm it produces 40 eggs/day, when it reaches 6 mm 70 eggs/day, and so on.

- When the generation period is known, all the halibut are treated so that all live flukes fall off and die.
- The water must also be treated because free-swimming flukes may occasionally be observed.
- The eggs are normally in the sand between the grains and survive the formalin treatment.
- The treatment is repeated before the first surviving parasites (which were eggs during previous treatments) may have reached 4 mm in length.

For treatment of halibut flukes, a somewhat stronger formalin concentration (1:2,000) is usually used.

Treatment of sores

Sores can and will occur on reared halibut. They are mostly caused by the halibut coming into contact with sharp stones, nets, tank walls and the like or by stroking. Sores often

first occur on the underside and they may therefore be difficult to detect. Sores also occur on the chin and the tail.

For small sores, **Bacimycin ®** or iodine solution (salt water) is recommended.

For larger sores, the use of antibiotics should be discussed with a veterinarian. Agents tested with good results are:

1 **Flumequine (Imeguyl)**

Bath: 25 ppm (25 g/m^3 water) for 2 - 3 hours.

Injection: 30 mg/kg body weight.

2 **Oxytetracycline (Terramycin)**

Bath: 10 ppm

Injection: 10 - 12 mg/kg body weight.

46.

Eye damages.

Some fish-farming facilities have experienced some eye damage among halibut. The following symptoms have been observed:

- One or both eyes become partly filled with gas. It is easily identified because the foam in the separation between gas and liquid is visible, and the eye often "swells" in size.

- Paling of the tissue in the eye, the lens, among other things.

- Collapse of inner parts of the eye.

- The eye(s) fall out, resulting in a bloodied sore(s).

It is important to note that, in some cases, the condition of having gas in the eye may be a phase which is reversible, that is, the eye may return to normal. In other cases, the condition remains stable or deteriorates. The latter results in several symptoms, resulting in the halibut becoming blind.

Blindness in one eye need not represent any problems as such fish can still take up nutrients. Blindness in both eyes, on the other hand, will present a great problem because the fish must be helped with feeding.

Gas in the eyes has been observed in wild caught halibut and in halibut hatched in rearing experiments. This means that it is not the capture that causes the problem. There are several theories about the cause of gas in the eyes but, so far, none of them have been proven. The theories extend to areas such as gas supersaturation, insufficient water pressure, parasites, fungi, formalin treatment (not likely), effects of light, mechanical wear and tear, biting, and so on.

As previously mentioned, this phenomenon has not been observed in halibut kept in five- (5) metre deep tanks. Of 12 brood halibut which were moved from a tank having a depth of 1.5 metres to a tank 0.9 metre deep, 4 individuals developed gas in the right eye in the course of 2 months. The theory about insufficient water pressure is therefore plausible and will be tested in experiments.

Anaesthetics.

During stroking and handling where the halibut is lifted out of the water, it often begins to slap after five to ten seconds. Anaesthetics can be used if it is too difficult to handle the fish or there is danger of the fish (or personnel) being hurt.

Agents which have been tested with good results: **Benzocain** and **Metomidat. Vet.** (50 mg/ml) are recommended.

When using **Benzocain**: 4 ml ("one cap") per 10 litres of water should be used. Benzocain increases in strength in water over time and the prepared anaesthetic solution should therefore be changed at regular intervals. Monitor closely how quickly the fish falls asleep and never trust that just because yesterday's mix ratio was right yesterday, it is therefore likely to be right today.

Benzocain is used in saturated solution with alcohol, that is, white crystal precipitate shall be visible. When adding Benzocain to salt water, white flakes often float about in the water. These are precipitated crystals and are not harmful to the fish.

47.

Metomidat is not a traditional anaesthetic, but a soporific. It can be injected or used in a bath. The preliminary recommended dosage administered by intramuscular injection is 5 mg/kg halibut, intravenous 2.5 mg/kg (at a water temperature of 8 - 10 ^{o}C) (Mattson and Rønnestad, unpublished). When used in a bath, 5 ppm is considered a sufficient dosage to effect a quick anaesthesia of cod (N. Mattson, pers. comm.) This is probably also appropriate for halibut.

REPRODUCTION AND SPAWNING.

by

Ivar Rønnestad

and

Elin Kjørsvik

Introduction

From the first rearing experiments in 1974, all experiments carried out on fertilized halibut eggs were based on eggs and milt from fish newly caught out on the spawning grounds (Solemdal et al (1974), Lønning et al (1982) and Blaxter et al (1983)). This method produced poor quality and quantity and the lack of an assured supply of eggs was an impediment to further progress. Since 1982, several institutions and firms have developed their own broodfish populations in order to have a guaranteed supply of eggs.

Stroking was, however, continued and this has been the dominant technique to obtain fertilized eggs up to the present. At some facilities, experiments have been carried out with natural spawning, but with no success. Both methods are dealt with below.

The halibut is a so-called portion-spawner, that is, it spawns

several times in the course of the season. This is in contrast to the salmon which only spawns once in a season. In captivity, the halibut produces mature eggs up to 15 times in the course of a season and at two to eight days intervals (K. Hamre and I. Holmefjord, pers. comm.). The volume of eggs produced by different fish varies from less than one litre to more than 20 litres. Each litre contains about 40,000 eggs (See also "Nutrition and Reproduction", page 16).

The season varies depending on location, but at the Aquaculture Station at Austevoll the main part of the season is from the beginning of February to the beginning of April. Fish caught near the Faeroe Islands have their main spawning period in the middle of June and some fish still produce eggs at the end of July (I. Fjallstein, pers. comm.).

Experience from MOWI shows that it takes a couple of years before newly-caught broodfish produce eggs in captivity (K. Hamre, pers. comm.). At LMC, on the other hand, a relatively newly caught halibut produced 3 litres of eggs of good quality (W. O'Connell, pers. comm.).

First-time spawners have eggs of very poor quality, at least when produced by stroking, even though the amounts may be acceptable.

Female halibut may be stroked several years in a row. At Akvaforsk, three females were stroked for the fourth season in 1988. The spawning rhythm changes somewhat from year to year (I. Holmefjord, pers. comm.). At MOWI, on the other hand, every fish has the same rhythm and the season starts at the same time every year (K. Hamre, pers. comm.).

49.

Stroking

Stroking simply consists of pressing carefully (ripe eggs come out easily) on the belly of the fish with a stroking motion towards the genital opening so that eggs or milt are forced out (See Figures 11 and 12). This can be done by

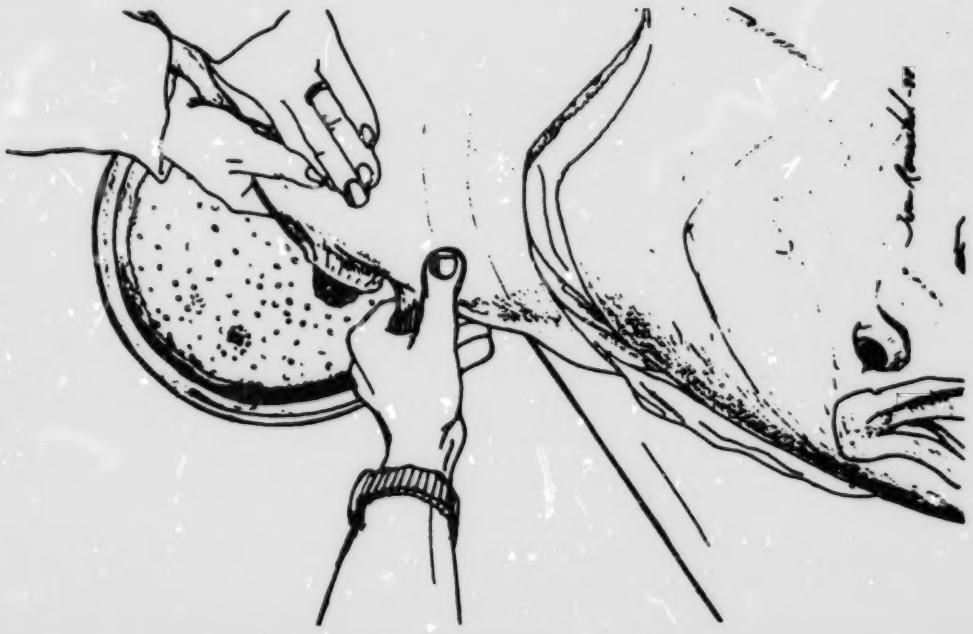


Figure 11. Stroking of a female fish.

stroking with the hands on the upper side only or on both the upper and lower sides of the fish. It is very important not to stroke too hard. Experience has shown that this results in damaged eggs and, more seriously: to bloodied and infected oviducts. The first indication of internal damage is bloody egg liquid. This was a problem in the beginning when stroking brood fish and it was normal to lose several fish in the course of one season (Anderssen, 1985).

Figure 12. Location of gonads and direction of stroking of male and female halibut. In this figure it is evident that the female has a slightly longer gonad than the male. It is therefore not necessary to start the stroking as far back on the males as on the females.

Stroking and fertilization

The following is the stroking procedure followed at the Aquaculture Station at Austevoll during the 1988 season:

Start with the Female fish

- **Lift the female fish out of the water.** This is easiest when using a canvas sail (see page 21) or a mechanized stroking table (see page 110).

- **Spray with fresh water around the genital opening.** This may not be necessary but it provides better hygiene.

- **If the eggs do not come by themselves or by slight stroking, then the fish should be returned to the tank.**

- **Carefully stroke the eggs out into a dry bucket.** Make note of the female fish number. The ventral fins should be lifted out from the body so that the eggs can run freely. The eggs will not be damaged even if a little sea water gets into the bucket as long as one does not wait too long, preferably no more than five minutes, before fertilizing. To varying degrees, some clear egg liquid comes with the eggs. It has not been investigated whether its volume or composition is of any significance to fertilization.

- **Put the cover on the bucket.** This is important because the eggs cannot tolerate light. The cover also protects against bacterial attacks.

- **The eggs must be stored cold until they are fertilized.**

- **Repeat the preceding steps for all female fish.** One bucket should be used for each female. This will ensure proper follow-up of each egg group and the monitoring of the percentages of fertilization and hatching. As well, it will make it possible to determine which females produce good and which produce poor eggs. The fertilization percentage is also dependent on the quality of the milt from the various males which are used.

- **Stroke the milt from the male.** An ordinary litre measure is adequate. Start collecting the milt when it comes out in a stream. If the milt is collected when it runs along the skin of the fish, a number of bacteria as well as sea-water will come along involuntarily. A syringe or a beaker can be used.

- **Carefully mix the milt from two males with sea water.** Sea water activates the milt. Activation means that

the sperm, from being tightly packed and immobile, become mobile and swim in search of eggs. The normal ratio between eggs and milt is about 100:1.

- **The milt is added to the eggs immediately.** The sperm are not likely to be active for more than five minutes.

- **Thereafter, add more sea-water.** The total volume of water should be from three to ten times that of the original volume of eggs. Stir carefully. Put cover on.

Some hints

- In some cases, the female does not release the eggs by slight stroking even if the belly is very swollen. There may be many reasons for this:

  1. The eggs have not ovulated (They are ripe, but have not released from the follicle cells). In this case, leave the fish for the time being and take it up again later on. Such frequent treatment stresses the fish and it should not be done too often.

51.

  2. The fish tightens its musculature, probably due to stress, so that even if the eggs were ovulated (loose in the gonads) they are not expelled. One

can wait a little while and see if the fish relaxes. If this does not work then, also in this case, the fish must be returned to the tank.

- Even though one may want to wade in the tank, constant changing of the water level should be avoided. It is then probably better to maintain a constant low water level (80 cm) so that one can enter the tank whenever desired.

- Those who stroke the fish should handle it as gently as possible and with calm movements.

- The halibut is normally very calm as long as it is in the water and there is no problem getting hold of individual fish. One can guide the halibut carefully with one's feet.

- At least three persons should be present during stroking; one to hold the bucket and lift the ventral fins, one to monitor the fish and one to stroke it.

- Avoid rapid changes of light conditions. Shaded lighting which can be adjusted is the best. Photography using flash is not recommended.

- The halibut should be used to contact with humans, otherwise the stress in connection with stroking will likely increase.

- Avoid all unnecessary visits outside the regular tending periods and during stroking.

Hygiene

- It is important that the water used in connection with the fertilization be as fresh and clean as possible. All bacteria collected during stroking create problems in the later treatment of the eggs.

- All equipment used in the tank must be disinfected before use in accordance with the procedures outlined in the chapter on hygiene.

- An angular bucket or a bucket with a pointed pouring lip makes it easier to avoid getting urine and feces from the fish in with the eggs. The urine is probably quite harmless since it does not contain bacteria and also has the same salinity as the egg liquid. (A.M. Jensen, pers. comm). The feces, however, contain enormous amounts of bacteria which may settle on the eggs and later destroy them.

During stroking, there is a certain amount of scraping off of

the layer of slime on the fish and sores are likely to occur as a result of tail slapping. Bacemycin, Iodine solution or the like, should therefore be available during stroking so that small sores can be treated immediately.

At several broodfish facilities, several halibut have died during or immediately after the stroking season, due to treatment in connection with stroking. It has been found that the material on which the fish is placed should be smooth and soft. For example, a thick layer of neoprene has been found suitable. Material such as foam rubber, which gives the fish too much friction and which sucks up liquid, must be avoided at all costs. Such material may effectively kill the halibut.

Another problem arises when the halibut is being lifted out of the water. When this happens, most halibut start to slap with the tail within five to ten second. Some, particularly small male fish, get "quite wild", while others slap with their tails with a strong but slow rhythm. These fish should only be controlled by being loosely held. After some time, the fish quiets down, in most cases. It is important to remember not to try to hold the fish by force since this stresses the fish even more. Slapping of the tail may result in bleeding wounds at the tip of the tail and, as previously mentioned, emphasis should be placed on a proper underlay.

52.

If the fish is so difficult to handle that it cannot be controlled, anaesthesia should be considered (see under "Disease").

Follow-up of the female fish

It is very important that every female brood halibut be monitored so that one can predict when there is the greatest probability for her to produce eggs. Various methods may be used to monitor the fish. At Akvaforsk in Sunndalsøra, the female fish is given the opportunity to release eggs in the tank two or three times before she is stroked at the next ovulation. This presumes that one can see which female released the eggs (she becomes thin). Using a flashlight, the eggs can be found relatively easily in the water (3 mm in diameter and glass-clear). This method presumes that the female fish has a rhythm of a certain number of hours between each egg ripening and that it has been constant over the period. This has been found to hold true for one season, but may change in the next (I. Holmefjord, pers. comm.). The method produces eggs of good quality if the stroking is carried out within a period of six hours after ovulation.

Another method is to stroke the fish at the first release of eggs. The advantage here is that one obtains all the eggs. The

disadvantage is that it may make the fish sufficiently stressed to develop an uneven rhythm, as described below, and that the quality of the eggs may be quite varied.

There are still no sure signs that a fish has ovulated the eggs and that the fish can be stroked. A good indication is, of course, the thickness of the female, but later in the season the thickness decreases with each egg group. Some people maintain that the ventral fin stands out from the body when the eggs can be stroked. However, opinions are divided on this matter.

Stroking stresses the fish to varying degrees so that the natural rhythm may be upset. In some cases the rhythm may be completely ruined and resorption of the gonads occur. How stress affects the spawning rhythm was demonstrated by a fish from which we took blood samples every day between two egg groups. This fish did not produce any new egg group that season. Also, in other fish which clearly were swollen it appeared that resorption of the gonads took place, perhaps due to the stress associated with stroking. It is therefore very important to handle the fish as gently as possible in connection with stroking.

Suggestions for building a mechanized stroking board.

This mechanized stroking board was built at the Aquaculture Station at Austevoll (see Figure 13). It was described by H. Rabben (1987). It eases the work of the personnel, particularly with heavy halibut, and reduces the stress on the fish.

53.

The board consists of an aluminium plate 2.2 x 1.0 m covered with a layer of neoprene 3 cm thick. The board is fastened to a balanced hanging device by means of a wire to a traverse crane.

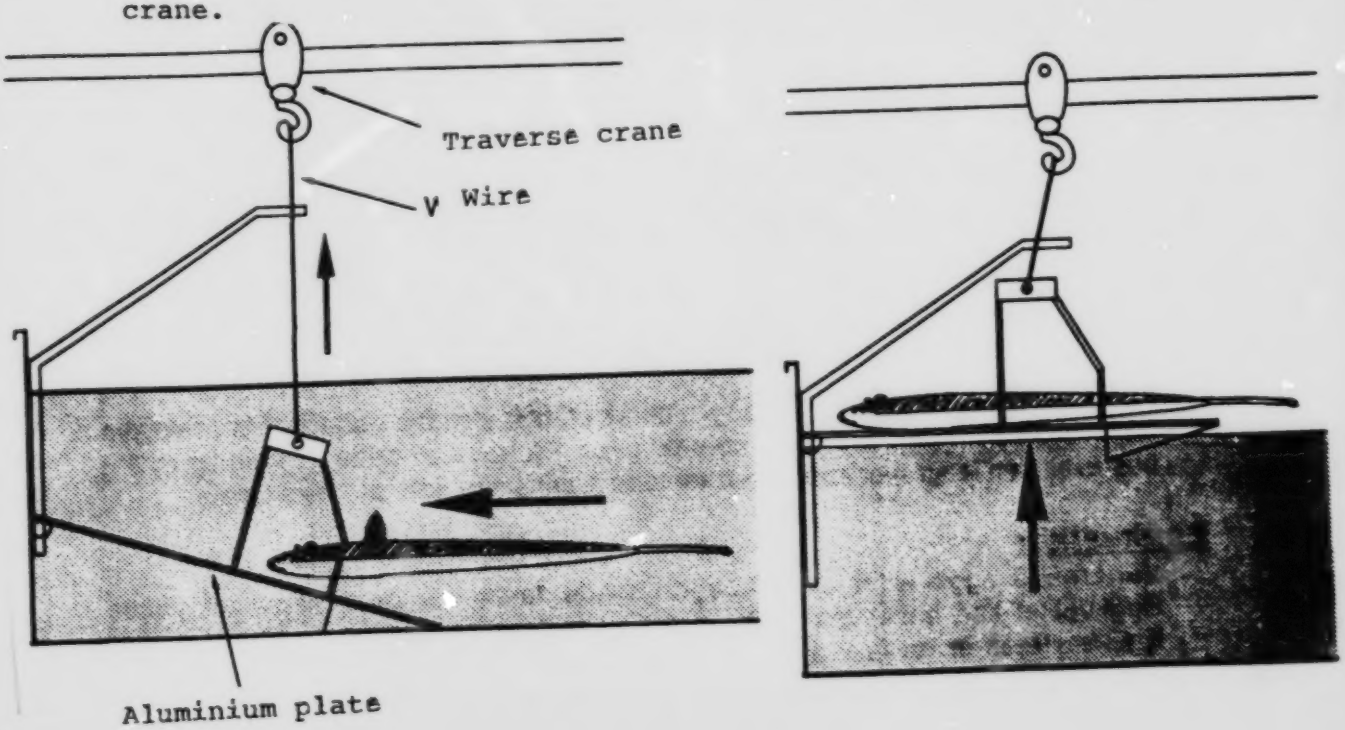


Figure 9. A mechanized stroking board.

When in use, the board is lowered into the water about 40 cm above the bottom. By lowering the board further an angular plane is created. The halibut is brought on to the board which is then raised to about 20 cm above the surface of the water. The fish can then be stroked and, at the same time, water can be prevented from getting into the bucket.

Natural spawning

When natural spawning can be controlled then that is far preferable to stroking. It saves both manpower and time and is much gentler for the fish. So far, two facilities have tried this, but without any significant amounts of eggs being collected in either the systems.

The working hypothesis on how to control natural spawning in a farming facility is that the halibut needs more space than is available in the five-to-seven metre tanks, and that the fish density must be very low. This is specifically based on a complex behaviour associated with spawning which requires a high volume of water. At the Lagoon Management and Construction (LMC), they keep only one female fish and three males in a 20-metre tank with two meters of water. (If the female fish produces 20 litres of good eggs, which equals 800,000 eggs, then that is considered acceptable production). The 1989 season saw experiments with natural spawning carried

out at the Sea Farm facility. It was not proven that the eggs were fertilized, even though the five females had periodic releases of eggs. The total amount of roe was 15 litres.

54.

At LMC, much work has been put into observing the behaviour of the fish in the tank at spawning time. The following behaviour can be observed (William O'Connell, pers. comm.):

When an egg group starts maturing, the female takes a spawning position in a depression. The males display great interest in the female during this period (about one day before ovulation), and they steadily make passes around the female halibut as well as positioning themselves on top of her. When the female is swollen, that is, hydration is completed, the males change their behaviour and lie and wait. The actual spawning and fertilization has, so far, not been observed.

When the results of natural spawning have so far failed to materialize, then this is partly due to problems of a practical nature which it is hoped to overcome. Despite this, natural spawning among halibut is a far more complicated method to master than that for cod, for example.

Egg quality

Varying egg quality is a limiting factor for the production of juveniles. Egg quality can be described as the egg's ability to

produce viable juveniles. Egg quality is established when the egg leaves the mother fish and fertilization has taken place, and effects of later treatment are not considered. Of course, this does not mean that the hatching results, etc., are unaffected by subsequent treatment. We know very little about how egg quality can be characterized, but morphological, chemical and physical characteristics could probably be used. It should be possible to employ practical criteria for good egg quality as early as possible in the development of the eggs - and they should preferably be as simple as possible to use.

Criteria for egg quality

Few studies have been carried out on the quality of halibut eggs. In most of the studies (other species), the fertilization and hatching percentages have been used as criteria for the egg quality. The fertilizing capacity is useful when determining poor egg quality, but this is not sufficient to guarantee a good development and survival at other stages.

Morphological properties are more sensitive than surviving. Good eggs are glass-clear and have even, round shapes. They have well delineated and symmmetrical early cell divisions; these cell divisions are poorer in eggs with poor survivability. Poor eggs may be irregular in shape, and dead eggs have a yolk mass that is off-white or milk-white (white dot in the egg).

Physical and physiological properties: Poor eggs are usually heavier than good eggs and therefore do not float as well. This may be related to the eggs' osmo-regulation. The ability of an egg to withstand mechanical stress (egg strength) can also be poor in poor egg groups. Egg diameters vary somewhat from fish to fish (3.0 to 3.3 mm, I. Opstad, pers. comm.), and in the course of the spawning season. There are no main points to indicate that egg size is of any significance to egg quality, but it appears that the first eggs spawned during a season are poorer than those that come later.

Chemical properties have not been studied for halibut, but it is possible that the egg's content of inorganic components, lipids, proteins and amino acid composition can reveal something about quality. This will also be studied. Content of pigments is probably of no significance to the quality.

55.

Chromosomal defects occuring in the fish egg are often deadly and the appearance of such defects is probably a sensitive indicator to describe egg quality.

Factors of significance to the quality.

Over-ripening and storing of eggs: To achieve a good survival rate, it is important to fertilize the eggs within a certain period of time after ovulation or they become over-ripe. One

of the important tasks in relation to egg production should therefore be to chart the ovulation cycle of spawners, and the survivability of the eggs during the period after ovulation. This period varies for the different species and is temperature-dependent. Among the halibut it appears that the eggs should be fertilized within 12 hours after ovulation (I. Holmefjord, pers. comm.), but several studies will probably be needed to verify these results. It may often be appropriate to store unfertilized eggs cold (refrigerated) in the egg liquid after stroking and before fertilization, since they appear to undergo changes similar to those in the ovary under such conditions. (I. Holmefjord, pers. comm.).

Over-ripening is generally shown as a morphological "degeneration" in the fish egg; the egg becomes more opaque or more discoloured, cortical alveoles aggregate (melt together) and the cytoplasma takes on more disorderly appearance. However, it is important to remember that the changes in the ability to survive take place before the morphological changes become apparent. Changes in the egg, due to over-ripening, may be due to a proteolytic breakdown of, among other things, the yolk proteins and the loss, through the egg membrane, of small organic molecules such as amino acids and peptides.

Broodfish nutrition and the enviroment are also significant to egg quality. However, this has not been investigated for halibut. The

biochemical composition of the eggs generally has a clear relationship to the composition of the feed for the spawners and, among other species it has been proven that the proper amount of, for example, essential fatty acids is very important to the survivability of the progeny. Different sources of protein, the level of protein and content of minerals contained in the diet are also likely to affect egg quality. The temperature during gonad maturation and spawning is also likely to affect both fecundity and egg quality.

Milt quality

The milt, or the sperm, may also be of varying quality. It can be roughly evaluated according to appearance. Milt of good quality is milk-white; milt which contains a fraction of clear yellow liquid is poor milt.

Milt normally flows evenly, even though occasionally it may flow a bit lumpy. This does not appear to be of any significance to quality (K. Hamre, pers. comm.).

To be certain that the milt is of good quality, the sperm should be looked at under a microscope. When seawater is added, the sperm are activated, it "teems with life", if the quality is good (motility test).

At the end of the season the milt often becomes more viscous,

that is, thickens. At this time (May - June), the males can often be observed emptying milt in the tank.

56.

Cryopreservation

A labour-saving alternative to stroking the male after each stroking of the female, is cryopreservation of milt (Cryos = frost, preservation = storage). The advantage of this method is that it is only necessary to stroke the male once per season.

The method for halibut is described (Bola et al, 1987):

- Stroke the male for the milt. The milt is collected in syringes to avoid contamination.
- Check the motility of the milt (see prev. page).
- Transfer the milt to petri dishes placed on crushed ice.
- Dilute the milt (ratio 1 : 3) with a NaCl solution which contains glycerol to prevent frost damage.
- Distribute the diluted milt in small holes on a dry ice plate which has been made up in advance.
- Store the samples in liquid nitrogen.
- Thawing of the sample is quickly accomplished using a Ringer solution which maintains a temperature of 40 ^{o}C.

Manipulated spawning period

As for other species, it is probable that the spawning period can be altered, either through manipulation of the light conditions or by hormone treatments. Experiments are in progress to clarify this.

FROM EGG TO JUVENILE

Introduction

by

Ivar Rønnestad

The production of juveniles appears to be the greatest bottleneck in halibut production. The first two juveniles at the Aquaculture Station at Austevoll were produced in 1985 (1980 in Flødevigen). In 1988, about 1500 halibut juveniles were produced on land. This figure does not represent any clear break-through and a massive broad research effort is still required before the first life phase is mastered. So far, most of the research has concentrated on the storage phase (from newly hatched larvae to start-feeding) but, as a relatively high survival rate up to start feeding has been experienced, the problems involving start-feeding have been brought more into focus.

This chapter sums up the status of knowledge about halibut juveniles. There is great uncertainty attached to some of this material and the next few years may prove to bring, among other things, great changes in the understanding of production of juveniles.

In Figure 14 below, an overview of the first part of the life

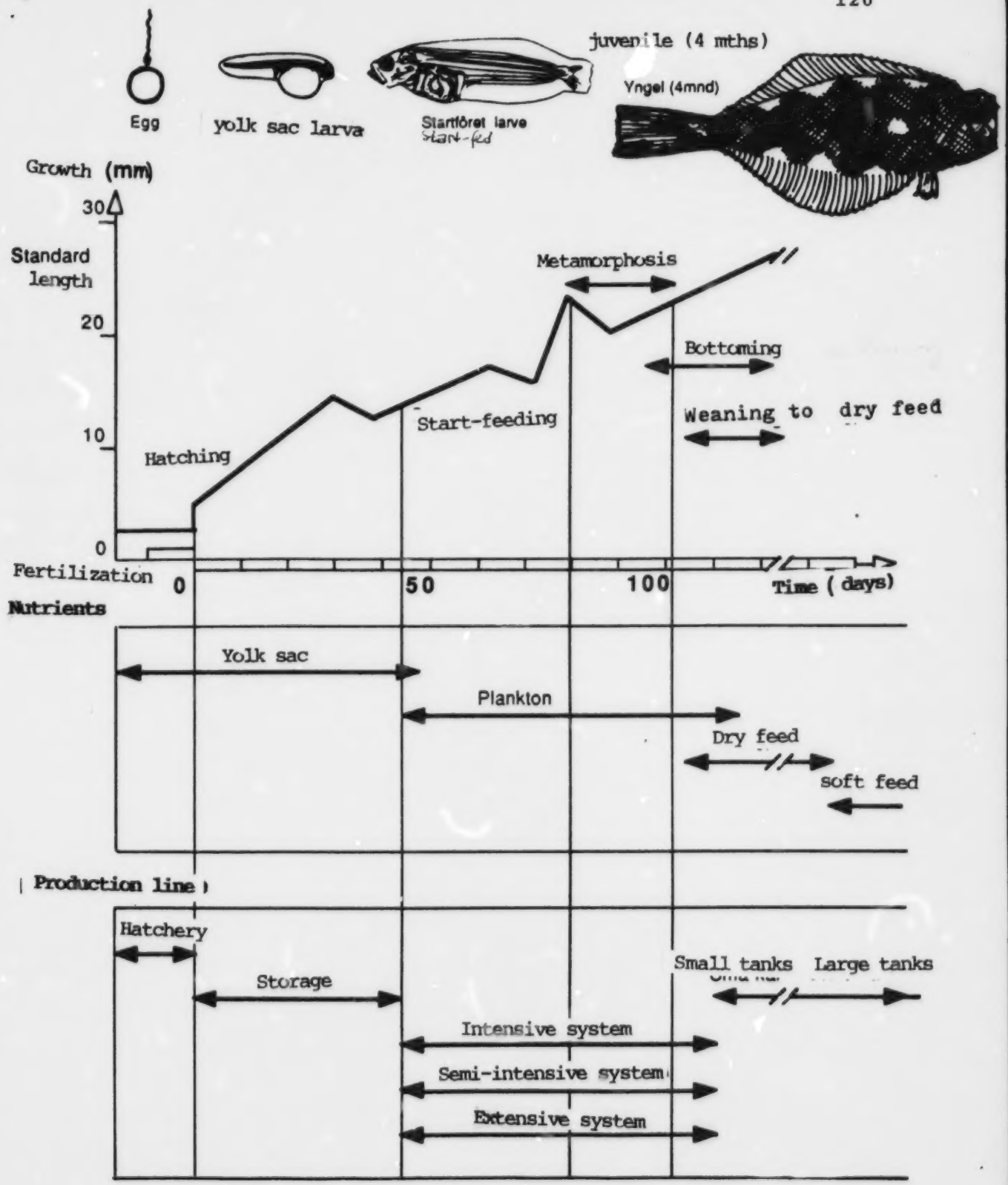


Figure 14. First part of the life of a reared halibut (see text for explanations). The growth curve and drawings of larvae are taken from Pittman (1987).

of a reared halibut is presented. It may be useful to refer to when discussing various details in the course of the life of the halibut.

In this chapter, the term "day degrees" is used. This is common in salmon farming and is based on the fact that the development at a given stage can be predicted based on a linear relationship between water temperature (^{o}C) and the number of 24-hour days since hatching. It is important to note that the biological background for this statement (Q_{10}) does not permit linearalization of this relationship at too great fluctuations in temperature. The reason for this is that the rate of a physiological process will increase as an exponential, and not linear, function of the temperature. Furthermore, the variations in water temperature are also a source of inaccuracy.

EGG PHASE

The Biology of the Egg

by

Anders Mangor Jensen

The earliest phases

Immediately prior to ovulation, the egg has gone through the so-called final ripening in the ovary of the fish. All substances which the egg needs to develop to a larva ready for start feeding should be incorporated in the course of this phase. At this stage, the egg is called an oocyte (see also "Nutrition and reproduction", page 16). At the same time, a porous egg shell has been formed around the egg, which before spawning is soft and flexible. The egg shell is often called Chorion or **zona radiata** (see Figure 15) and consists of structural proteins, not unlike those found in hair and nails of humans, among others. The egg shell is not a part of the living egg, but serves to protect the egg against physical stress. At this stage the egg consists of only one cell but, then, the egg cell is one of the largest cells known (halibut eggs are about 3 mm). However, most of the cell volume is filled by a large central vacuole which contains the yolk mass.

The cytoplasma with its organelles, lies as a thin layer around the

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perimeter of the egg cell. In the cytoplasma layer one can also find the so-called cortical alveoles. These are small and spherical and also contain various organic components which are of great significance in the so-called activation proccess. A number of physiological changes take place when the egg is activated. The most visible of these is the formation of a space between the egg cell and the egg shell. This space is filled with sea water which penetrates the pores of the egg shell. It can also easily be observed that the cortical alveoles have taken a more peripheral position.

60.

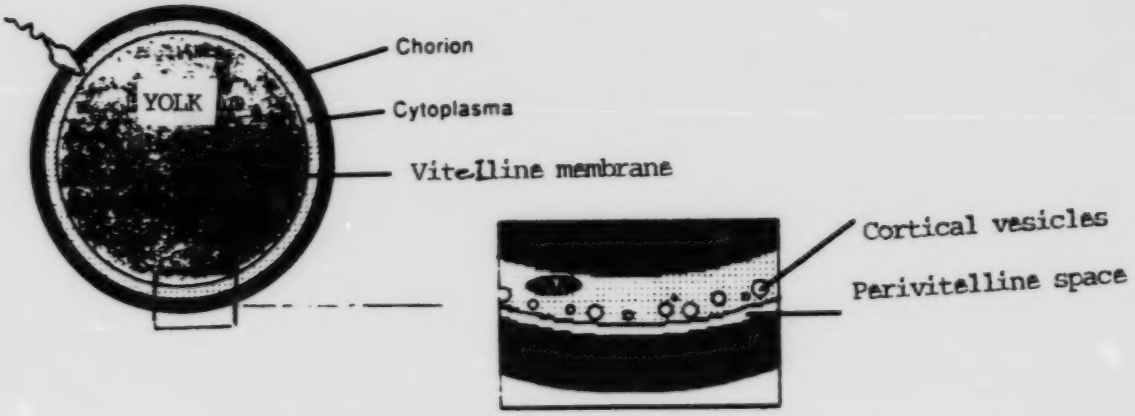


Figure 15. Halibut egg. See text for explanation.

Fertilization

The fertilization of the egg takes place when the sperm cell transfers its genetic material to the egg thereby forming the zygote (merging of the male and female chromosomes). The sperm comes into contact with the egg cell through an opening in the egg shell which is called the micropyle. This opening is just big enough for one spem cell to fill it. In this manner, the opening is effectively blocked to other sperm. Immediately after fertilization, the cytoplasma starts to move along the periphery of the egg. Some hours later an elevation in the animal pole of the egg has been formed. This area, which consists of cytoplasma, is called a blastodisc or germinal disc at this stage. After a further few hours, the germinal disc starts to divide into increasingly smaller cells. The stages of development of the foetus during this period are named according to how many cells they contain (for example, the 16-cell stage). Along the edge of the germinal disc (which is no longer called the germinal disc once cell division has started), a ring of flatter cells is formed. This tissue is called the periblast, and will finally form a complete tissue around the yolk sac. This process of growing around is often called the "Epiboly", which means 'growth around'.

After activation/fertilization, the egg is free-floating inside the egg shell. It can easily be observed under a magnifying glass by

rotating the egg a little. Almost immediately, the foetus/germinal disc orientates itself downwards ("in the bottom of the egg") due to its density being higher than the water surrounding it.

Pelagic egg.

The halibut spawn pelagic eggs, that is, eggs which float/drift freely in the water mass. For the egg to sustain itself in the pelagic waters it is important that it has a density not higher than the medium in which it may, at any time, exist. When an egg is spawned it will contain about the same concentration of salts as the body fluids of the female fish. This means that the concentration of salts will be about 1/3 of that normally found in sea water. A large volume of diluted sea water in the yolk sac will therefore give the egg an

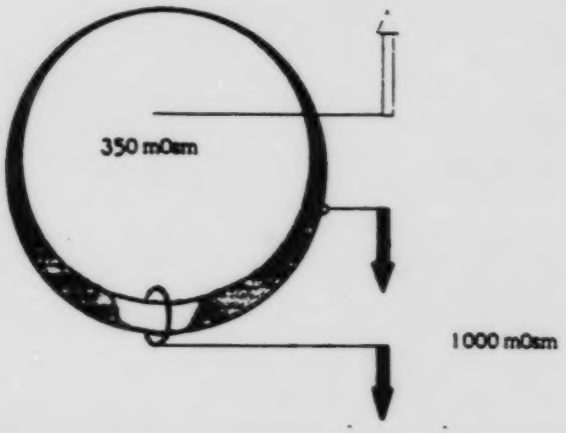


Figure 16. Forces which act on the pelagic halibut egg.

upward buoyancy (see Figure 16). On the other hand, both the egg shell and the growing foetus are heavier than sea water and will contribute to pulling the egg downwards.

It is of vital importance to a pelagic egg that the upwards buoyancy be greater than its weight. In very deep waters the eggs may sink some kilometres without hitting bottom, while in a rearing situation this is one of the greatest problems. If a pelagic egg remains lying on the bottom of the incubator, it will be quickly attacked by marine bacteria and die. Heavy eggs have been a problem in connection with rearing halibut. Some researchers maintain that buoyancy (or flotation) is a good measure of egg quality among pelagic eggs (see "Egg quality", page 112).

61.

Osmo-regulation

When the egg is spawned from gonad to saltwater the external environmental conditions will change drastically for the egg. In the same manner as among adult fish, the body fluids of the egg will have a salt concentration of 1/3 of that found in sea water. As in the case of adult fish, the egg will therefore be exposed to the physical process called osmosis. Osmosis acts to equalize the differences in concentration between the fluid-filled volumes which are closed off from each other by membranes. If this process were allowed to work, the egg would shrink due to loss of water to the surroundings. This special problem is circumvented by the surface membranes being water

tight. The membrane (not the egg shell) which separates the foetus from the sea water is the tightest known (actually it is almost 1,000 times tighter than the next one on the list: the urinary bladder of the frog).

Despite this, it is impossible to have life in complete isolation from the surroundings. All living organisms will, to a certain degree, exchange substances, for example, respiration gases or nutrients, with the external medium. An egg is self-sufficient in nutrients, but to maintain a stable inner environment, oxygen must be supplied and ammonia and carbon dioxide expelled. These exchanges occur by diffusion through the animal's surface membranes, so that a certain permeability must exist. Because the membranes are not completely tight, the egg will continuously lose water to the surroundings. This can be observed as an increase in the space between the egg shell and the foetus, because the diameter of the egg shell remains constant. The halibut lacks the ability for active osmo regulation before it has hatched. Therefore, it cannot, like the cod, replace the loss of water through the uptake of salt water and expel excess salt through the chloride cells.

Respiration.

In contrast to the adult fish, the egg has no specialized respiratory apparatus (gills and gill cover pump), and all respiration occurs by diffusion over the surface membranes. This is a well-known phenomenon

among small evertebrates and is conditional upon the animal's effective diameter not being greater than ca. 1 mm. Diffusion is a relatively slow process so that the surface volume relationship to the animal decides whether it shall "base" itself on this type of gas exchange. A small animal has a large surface in relation to its volume. A halibut egg has a diameter far greater than 1 mm, while the diameter of the foetus itself is far smaller, and it is this which, first and foremost, needs oxygen. In addition, the foetus also has a very low metabolism at this stage, so that the need for oxygen is very limited.

Even later in the development, when the larva is close to hatching, there are no other specialized respiratory surfaces (gills). However, at this time there exists a functional circulation system (see also "Biology of the Larva"), notably without hemoglobin, which can first be observed after metamorphosis.

62.

Hatching

Hatching takes place through the freeing of a hatching enzyme from special secretory cells. These enzymes break down the egg shell from the inside so that the larva can easily escape. Among halibut, hatching takes place in accordance with a special system (Helvik, 1988). Here, hatching glands are located in a ring around the head of the larva (see Figure 17).

When secretion of the hatching enzymes starts, they will cut the "cap" off

the egg in the best breakfast-table manner. Experiments have shown that if there is too much manipulation of the eggs during a critical period, the larva will have difficulty in breaking out of the egg shell. This is probably related to the hatching enzyme being distributed around the whole perivitelline space and thereby losing its localized effect on the egg shell (Helvik, 1988).

The difference between the newly hatched larva and the egg just before hatching is, in reality, only the egg shell. No dramatic physiological

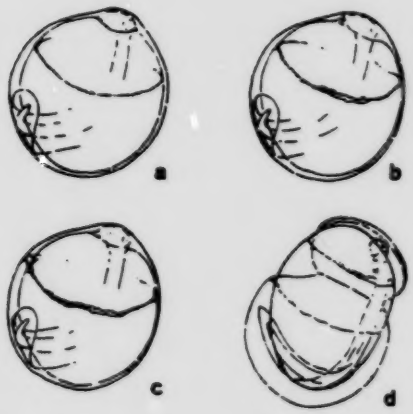


Figure 17. The halibut larva escapes from the shell.

a) The egg shell cracks ventrally to the yolk sac.

b) and c) The crack breaks up towards the larva body.

d) Periodic muscle movements force the larva out.

The figure is taken from Helvik, 1988.

or anatomical changes occur in the larva at time of hatching. At the time of hatching the larva is freed of what up until now was "the sinking portion", that is, the egg shell. The result is that the larvae suddenly becomes lighter and will rise in the water. Ecologically, this can be related to the fact that the larva, for first feed intake (start-feeding), must be in the photic zone of the water, where animal plankton are found. In addition to the yolk mass as an hydrostatic buoyancy organ, the larva has developed large fluid-filled spaces along its whole body. These are called subdermal spaces due to their location on the larva. The spaces are filled with normal body fluids and will therefore provide buoyancy in sea water.

Yolk Sac

The contents of the yolk sac consist, as previously mentioned, mainly of water (96 %), but it also contains the nutrients needed for the larva to develop to the stage where it is able to take feed from its surroundings (exogenous nutrient intake).

63.

In the pelagic eggs, the endogenous nutrients are present mainly as dissolved molecules. This causes the yolk sac to be transparent (see also under "Behaviour of Larvae", page 151). The uptake of nutrients probably takes place by diffusion of molecules in the liver, for later distribution through the blood. In contrast to, for example, the salmonids, larvae of pelagic eggs have no vascular system enclosing the yolk mass.

Construction and Operation of a Halibut Hatchery

by

Anders Jelmert

Introduction

In the wild, halibut eggs are likely to be at relatively great distances from each other for a period of time after spawning and the fate of a single egg will influence the other eggs from the same batch of spawn to a small degree.

For practical and financial reasons, it is necessary to store the eggs at far greater densities in a hatchery than in nature. The eggs will therefore be more exposed to bacterial influences and when an egg dies this could greatly affect the rest of the batch due to increased bacterial growth.

This can be illustrated by the following example:
A halibut egg contains about 700 ug of carbon. If we assume that the egg shell accounts for 30 %, then we are left with slightly less than 500 ug of a very well suited bacterial substrate. A single dead halibut egg could then give rise to about 1,000,000,000 bacteria.

It is possible to compensate somewhat for this high storage density by increasing the exchange of water in the hatching incubators. However, this will result in increased stress on the eggs due to turbulence and mechanical stress.

Water and water quality.

The following properties are important.

- Relatively high salinity.
- Relatively low, and as even as possible, temperature.
- The lowest possible content of harmful micro-organisms.
- The lowest possible content of energy-rich organic material.
- The lowest possible particle content.
- Good oxygen saturation.

By using water from depths greater than 50 metres, it is often possible to find water which satisfies the requirements sketched out in the points above. Of course, consideration must be given to currents, proximity to possible muddy bottom, water exchange in deep pools, and so on.

Treatment of sea water.

I will confine myself to dealing with the dominant methods of treating sea water for hatcheries and the storage phase. However, more knowledge of this subject is urgently needed.

Filtration

In order to remove particles, step-wise filtration is often used. With present-day technology it is necessary to carry out filtration in several stages in order to avoid clogging the filtration medium.

Personally, I am of the opinion that particles down to ca. 1 um should be removed. However, there are no data which clearly **prove** that such extensive filtration is necessary.

UV treatment.

Transillumination of the water with light at a wavelength of 256 nm (ultraviolet light) will kill or inactivate most types of micro-organisms. The disinfecting effect depends on the dosage.

$$\text{Dosage} = \text{radiation intensity} * \text{time} \ (uWs/cm^2).$$

In many cases, it will be desireable to treat water with UV radiation.

As a general rule, it can be said that the shallower the water intake the greater the need to employ UV radiation. To avoid shadow effects, it is important for water which is UV treated to have been previously filtered. If there was a high density of micro-organisms at the begining, then the newly-killed cells will provide a foundation for better growth among those that survive and/or are introduced later.

Micro-organisms

The halibut is affected by micro-organisms throughout its lifespan. This somewhat special biology makes part of the lifespan particularly vulnerable. So far, problems originating primarily in bacteria have

been recorded. Some are directly pathogenic, such as, for example, some Vibrio species (see also "Disease" page 64). Others will harm the halibut egg/larva due to the processes in which they participate.

Example of such processes are:

- Consumption of oxygen.
- Breakdown of egg shell.
- Production of dihydrogensulphide (H_2S).
- Production of ammonia (NH_3).

It is important to remember that bacteria can grow rapidly given the proper conditions. In this connection, rapid means a doubling of the number in 1 - 4 hours.

Halibut hatchery.

Hygiene is a central element when planning and operating a hatchery (see also "Hygiene", page 61).

- The hatchery must be physically separated from the water systems for other fish, particularly broodfish.
- There must be disinfection stations/points which must be passed to enter the hatchery.
- Personal equipment, clothing, shoes, etc., only for use in the hatchery.

- Water: sea water from depths of more than 50 metres. Intake must be more than 2 meters from the bottom.

- Filtration: Coarse filtration. Alternative methods:
  - Pressurized sand filter
  - Gravitation filter, ca. 15-30 um.
  - Triangular filter.

  66.

  Fine filtration:
  - Cartridge filter with pore size less than 5 um.
  - It should be possible to back-flush (usually with fresh water).

- UV treatment, if there are doubts about water quality. The water should receive a dosage corresponding to the requirements for potable water, that is 1600 uWs/cm^2.

- If the chill is taken off water by warming it, then any supersaturation must be aired off.

- The hatchery must have two completely separate water supply systems so that one of the systems can be disinfected while the other continues to operate.

- The inside of the incubators must be as smooth as possible. Any release of gases from plastic hardening processes must be avoided.

- The lower portion of the incubators must be conical in shape.

- The water supply should, in most cases, come from the bottom, with an overflow at/near the top. A screening disc, which should be large, will prevent the eggs from going with the overflow.

- Supply valves to the individual incubators must be easily adjustable so that the proper flow for the individual egg groups can be set.

- It may be an advantage to have extra piping coming in near the bottom of the incubator to supply water with salt added.

- Some incubators may be earmarked for positive buoyancy eggs, that is, they have an alternative water supply from the surface.

Incubators

Egg groups which, for various reasons, have negative buoyancy must be given extra lift by creating a current pattern in the incubators which prevents too dense clustering of eggs. This is achieved by the design of the incubators (see Figure 18).

The current principle used for hatching halibut eggs is as follows:

Upwelling water in the incubators results in eggs with a specific gravity greater than water not sinking but remaining "afloat" in the upstream. The conical shape of the incubators has two functions. First, it will cause the water to flow at an uneven rate as it moves upwards. Second, this results in the eggs finding a point of equilibrium, which depends on their weight and the rate of the upwards flow of the water.

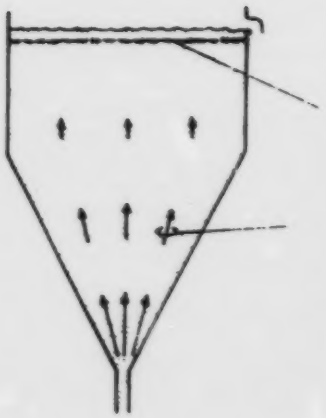


Figure 18. Sketch of incubator.

Operation/tending

There should be no more than 200 - 300 eggs per litre of incubator volume. 67.

Dying and dead eggs must be removed from the incubator as soon as possible.

The following procedure is adhered to when tending (see also Tending to silos, page 163):

- Turn off water supply.
- Add salted water (for example 40 ppt, up to 30 % of the volume of the conical part). This is added by a hose or a separate pipe in the bottom of the incubator.
- Wait until the desired amount of off-white/dead eggs have fallen down into the "plug of salt".
- Remove the plug of salt and dead eggs with a siphon or a separate short piece of pipe.

There will always be a certain mortality about 24 hours after the eggs have been placed in the incubator (from unfertilized eggs, among others). The incubator should therefore be inspected after day 1, day 2 and every second day from then on. If an egg group also shows high mortality on day 2 and day 3, then the group should be discarded. If any surface growth

is discovered on the eggs at an early stage (less than a week) after placing, the groups should be discarded and the incubator and other equipment disinfected.

Water exchange and bacterial growth

Incubators and silos often act as a continuous culture for bacteria.

If we have a more-or-less even supply of organic material (for example, even mortality rate for eggs or larvae) then bacteria will grow at a growth rate (u) corresponding to the thinning rate in the incubator.

$$u = D$$

The thinning rate D is defined as F/V (supply/volume). At an increasing thinning rate (greater F), the bacteria will not be capable of growing quickly enough after a while, and we will get a washing out of bacteria from the incubator.

This is a very important aid to operation of incubators and silos. An increasing water supply must be seen in relation to the flow and the turbulence in the systems.

Critical Periods in the Development of the Egg

by

Ingegjerd Opstad

Stress experiments carried out on halibut eggs from day 1 up to hatching show that the incubation period can be divided into several characteristic phases. The experiments were carried out at 7 ^{o}C.

Phase 1. This period lasts until Day 2, with a peak in mortality.

Phase 2. In this period, between day 2 and day 6, mortality is considerably less.

Phase 3. Day 7 after fertilization shows a new peaking in mortality. This coincides with completion of gastrulation.

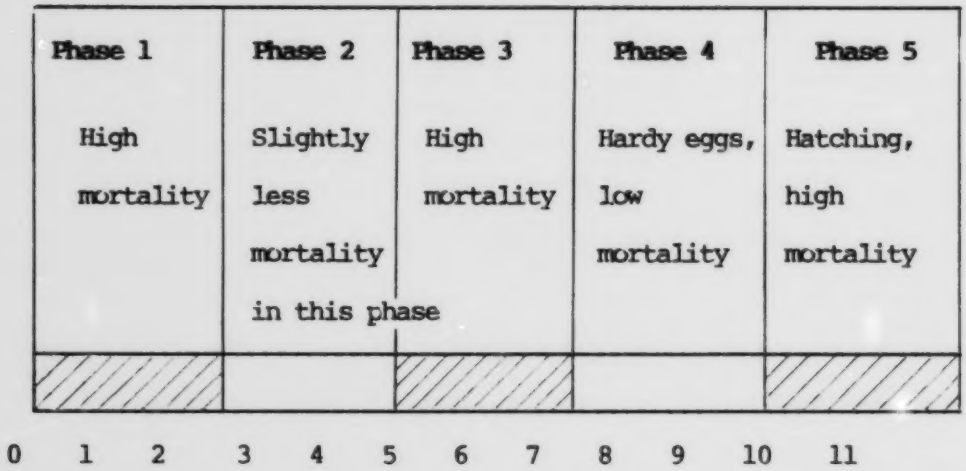
Phases 1 and 3 are two sensitive periods where water flow ought to be low and the eggs handled as little as possible.

Phase 4. From day 8 to day 11. Mortality is negligible during this period.

This period is well suited for handling the eggs, for instance, when transferring and shipping. During this period they can also tolerate high water flow, so that the period can be used to obtain a good exchange of the water in the hatching incubators.

Phase 5. From day 11. Hatching period. This is a very sensitive period with high mortality. Water flow should be as low as possible during this period. Preferably, the water should be turned off completely.

Table 9. Various phases in the development of the halibut egg.



STORAGE PHASE

Morphology of the Yolk Sac Larva.

by

Karin Pittman

Introduction

So far only one larva has been caught in the Northeast Atlantic (Haug et al, 1988). Knowledge of the early development of the halibut is therefore almost exclusively limited to observations in rearing experiments, at the Aquaculture Station at Austevoll, among other places.

At the time of hatching, the larva is 6 - 7 mm long and not much developed. The larva takes 8 weeks to absorb and burn the yolk sac content. In this period, the internal organs, pigmentation and a functional digestive system are developed. The period from hatching until the yolk mass is consumed is called the yolk sac or storage phase.

The development of the larva during the yolk sac phase.

On the basis of three seasons of halibut larvae production, we have chosen to divide the yolk sac stage into four morphological stages. In all these stages the notochord remains straight and the eyes

are symmetrically located on either side of the head. Up until the larvae reaches the exogenous feed uptake stage the growth rate is larger in standard length than in myotome height.

Stage 1. In this stage, which lasts from 4 - 7 days after hatching, the larva is relatively primitive. It mostly hangs passively head down in the water. The head is still pressed against the yolk sac and the eyes are almost semi-circular. At this time, it is <u>not</u> possible to observe pigmentation, drops of oil in the yolk, mouth, pectoral or abdominal fins, liver, gall bladder or red blood cells, but there is a primitive tubular-shaped heart. At this stage, the intestine consists of a straight tube. The larva drinks (Tytler and Blaxter, 1988), probably through holes in the gill region where the gills and gill cover will be formed later.

Stage 2 Duration 15 - 20 days. Many internal organs will be formed and the eyes will become pigmented. The eyes will change from having light pigmentation near the outer edge of the iris to becoming fully pigmented with the exception of a narrow band near the posterodorsal iris. The mouth develops from a small opening, but the jaw bones are not yet completely formed. The hole in the gill region expands to a larger gill opening. The larva begins to develop a functional digestive system. The intestine changes character from a straight tube to becoming a bit thicker at the front where rotation begins. Rotation

represents growth in the intestine which, in the end, will form a loop as the contents of the yolk sac are consumed. The intestinal loop will partly fill the abdominal cavity, but will not be completely formed at this stage. The liver is formed from a cellular mass on the left side in the forward part of the yolk sac. It can soon be identified as a separate organ, triangular in shape, which is attached to the yolk sac and the intestine. The gall bladder develops as a circular structure near the intestine on the medial side, and may be green in colour for a short period.

During this stage, the yolk sac will be reduced from elliptical to pear-shaped. The base of the pear is at the tail end of the abdominal cavity. The reason for this shape is: 1) Growth and rotation of the intestine in the left forward part of the abdominal cavity pushes the yolk back. 2) The pectoral fins develop from being small to becoming paddle-like fins resulting in a more streamlined forward portion of the yolk sac.

The heart changes from a primitive tube to an organ with four separate chambers. This results in the heart acquiring greater pumping capacity. Together with the heart's pumping capacity increasing, effective pectoral fins result in the larvae becoming more active. Now, it can reach a swimming speed of 3mm/second as opposed to 0.5 mm/second previously.

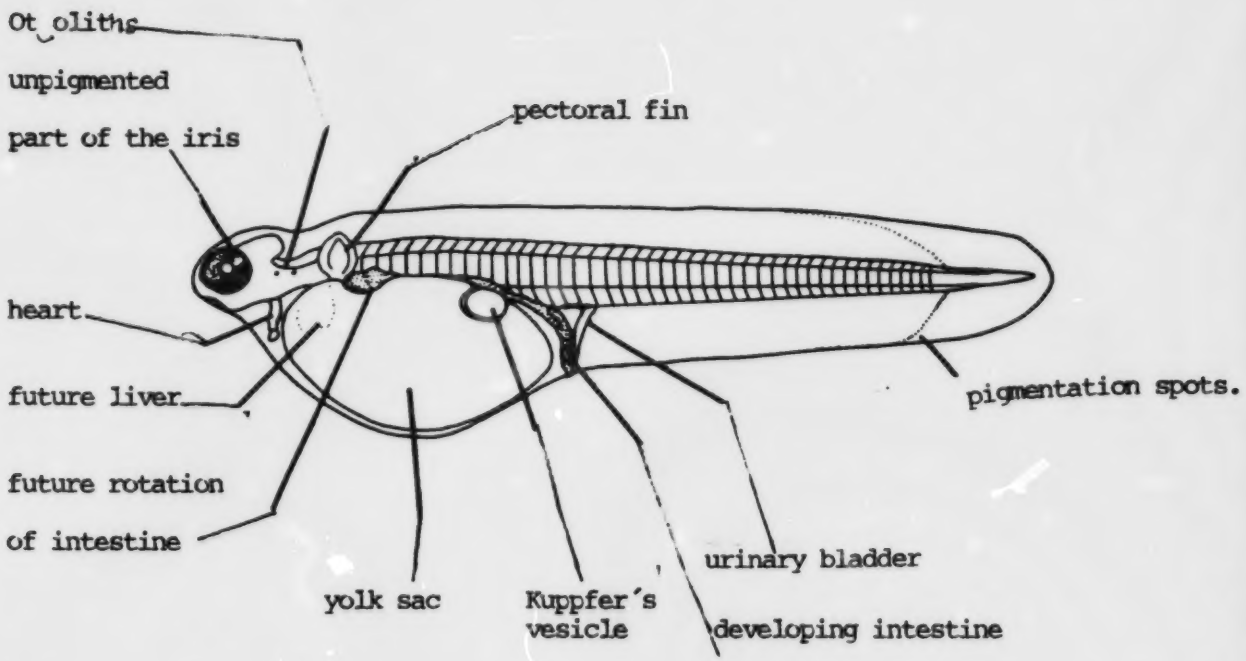


Figure 19. A halibut larva in the middle of stage 2. It has a length of about 12 mm (From Pittman et al, 1987).

Stage 3 lasts between 15 and 20 days. During this period, the formation of the internal organs is completed. The eyes become completely pigmented. The jaw bone and jaw joint calcify and the larva develops a functional mouth. The gill cavities expand further to form an opercular opening with naked gill arches. Even though there are only

a few gill filaments on the gill arches, the respiratory movements are normal. This means that the larva pumps water through the mouth and out over the gill arches.

During stage 3, the internal organs grow and become more specialized. The intestine forms a loop and fills the major portion of the abdominal cavity. The smooth musculature in the intestine thickens

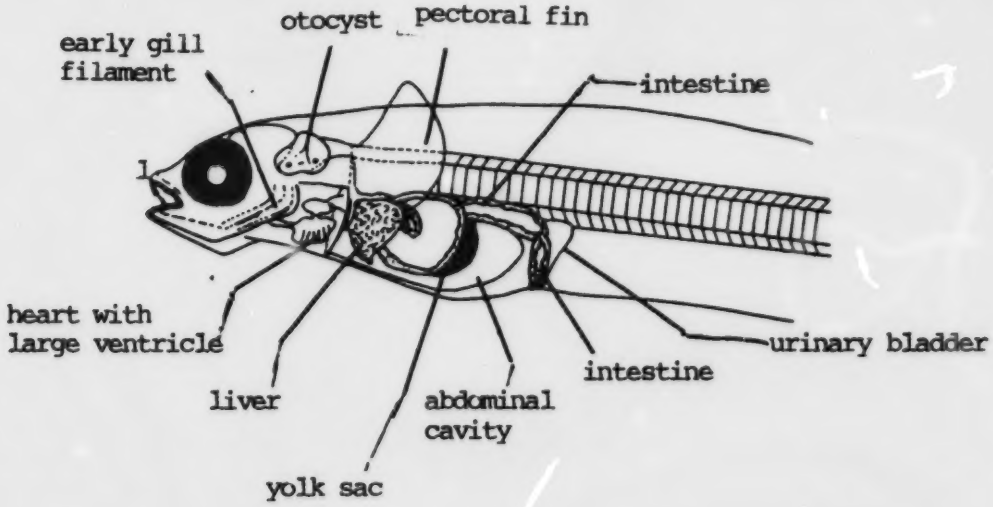


Figure 20. The internal organs which form during Stage 3 (From Pittman et al, 1987).

and, once in a while, a rhythmic contractile activity can be observed. The sphincterate (closing) musculature develops so that the intestine can be closed by the colon. As the intestine grows and the yolk mass

is consumed, the yolk sac takes on an elongated shape on the right side of the intestine. The yolk connects with the middle portion of the liver. The muscle tissue in the heart ventricle increases in thickness. The larva acquires a more streamlined appearance and it can now swim for up to a minute at a time. This is the stage, during rearing, when it is normal to introduce light and zoo-plankton to the larvae. 71.

Stage 4. This stage includes the last 5 - 7 days <u>before</u> the yolk mass is completely consumed and the larva begins to increase in height. This may happen simultaneously with start-feeding. Some black pigmentation spots appear near the ventral mid-line and along the

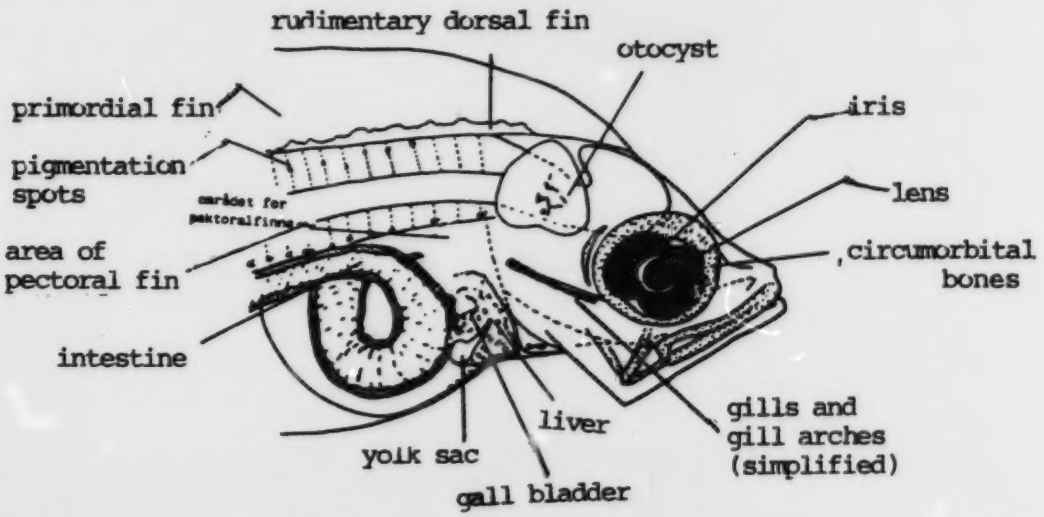


Figure 21. Stage 4 halibut larva found in nature.

(From Haug et al, 1988)

notochord. The gills continue to develop but will not be fully formed until later. The motions of the gill cover results in a flap becoming visible forward in the mouth. This flap acts as a one-way valve and prevents water from flowing back out through the mouth when the gill cover motion creates over-pressure in the oral cavity. A slight thickening of the tail muscles becomes apparent and the tail fin rays appear. Stage 4 is considered complete when the larva has begun its exogenous feed uptake and the myotomes increase in height so that the ratio between standard length and myotome height becomes less than 15.

72.

Growth and temperature.

The size and development of the halibut larva is temperature-dependent. At low water temperatures (3 to 4 ^{o}C), the larva takes a longer time to consume the yolk mass than at higher temperatures. On the other hand, at a given stage the larva will be significantly larger, due to more efficient utilization of the yolk. Furthermore, "cold water larvae" will also be more normally developed than if they were kept at a higher temperature.

There are some abnormal developmental traits in rearing. Gaping mouth occurs early in stage 2. This phenomenon results in the larva not being able to close its mouth. Oedema (accumulation of fluid) in the abdominal cavity can also be observed early in stage 2. This means that the abdominal cavity swells up and pushes the intestine up into a curve. This also results in the yolk mass acquiring an irregular shape

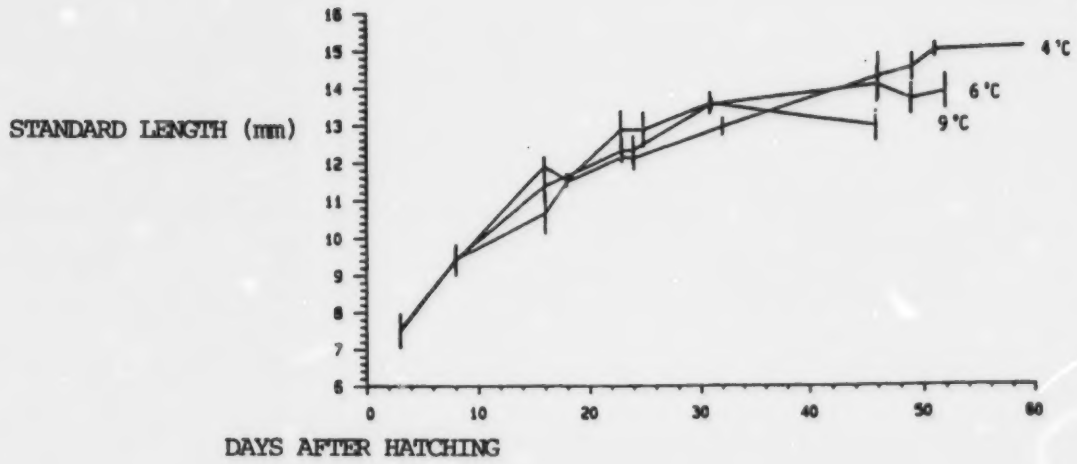


Figure 22. Growth of yolk sac larvae at 4, 6 and 9 ^{o}C up to and including stage 4. One egg group was divided into three where each group was exposed to the same temperature (ca. 6.5 ^{o}C) during the first week after hatching. (From Pittman et al, 1988).

and the heart developing improperly. During stage 3, these types of larvae can be found floating near or on the surface, normally on their backs. Both these deformities are related to too high water temperatures (Pittman et al, 1988).

Survival, mortality and causes of mortality.

All mortality data are given as number of dead larvae identified in the bottom water. This task is normally carried out every second day. This recorded mortality is not the same as the actual mortality for a given period of time, since some of the larvae may be degraded/decomposed in the system or they may float for a while before ending up on the

bottom to go out with the bottom water. No larvae with oedema have been found in the bottom water, probably because they float until they are degraded/decomposed. Deformities such as locked jaw and oedema will not necessarily lead to mortality before the larva has consumed the yolk mass and normally would have started its exogenous feed uptake, which they then cannot do. This can easily be misinterpreted as if the larva has problems with start-feeding. The real reason may, perhaps, be found as far back as the egg stage. The mortality curves may provide a certain insight into problems in various production systems. On the other hand, survival up to start feeding must not be mistaken for an indication that the remaining larvae necessarily are capable of eating prey. 73.

Measured in this way, the mortality in the yolk sac phase is also not the opposite of survival. Dead larvae may remain in the system until they have degraded (dissolved/decomposed), finally to leave with the overflow water or become sediment on the wall in the rearing system as nutrients for microbial activity. This is probably an increasing problem with increasing water temperature. The unrecorded mortality can only be estimated by comparing the number of larvae which were placed in the system, the number of live juveniles and the number of recorded dead larvae, somewhat difficult in practice.

The Behaviour of the Yolk Sac Larva.

by

Anne Berit Skiftesvik

The larva is quite passive during the first part of the storage phase (see Figure 23). It "hangs" head down in the water. When it is disturbed it swims actively for about 5 seconds (see Figure 24), again

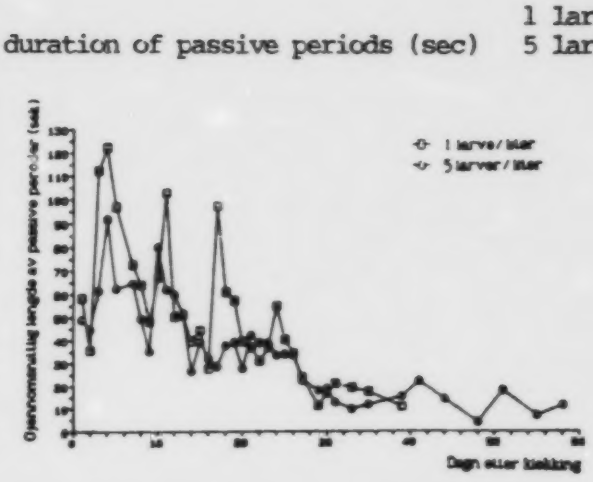


Figure 23. Average duration of passive periods for larvae, at two densities (1 larva/L and 5 larvae/L) from day 0 to day 39 and day 58, respectively. Each point is the average for 10 larvae (From Pittman et al, 1988).

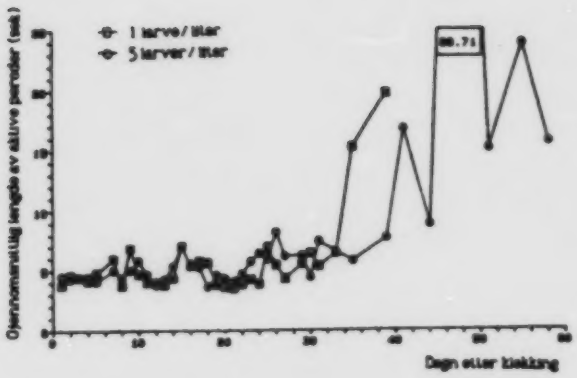


Figure 24. Average duration of active periods for larvae, at two densities (1 larvae/l and 5 larvae/l) from day 0 to day 39 and 58, respectively. Each point is an average for ten larvae (From Pittman et al, 1988).

to hang passively with the head down. At this stage, the larva is transparent and not very active, an adaptation making it not very vulnerable to predation in natural surroundings.

It is preferable for the larvae to remain quiet during the storage phase to ensure that most of the energy in the yolk mass is used for somatic growth and not burned through activity.

75.

Swimming

The swimming activity of the larvae is density-dependent. The duration

of passive periods decreases with increasing density. That is, swimming frequency increases with increasing larvae density. The reason is assumed to be increased contact with other larvae. In an experimental situation using an average larvae density of 5 larvae per litre and 1 larva per litre, the swimming rate was greatest at the highest larvae density. This may reflect a more stressful situation for the larvae. In a rearing situation, the larvae encounter many stress factors which they might possibly have avoided in nature, such as unfavourable light conditions, temperature, salinity and larvae density. Other stress factors which the larvae may experience in a rearing situation are noise, high bacterial density, transfers, etc..

Any disturbance leads to the larvae becoming active for a short period. The duration of the swimming period is about 5 seconds for larvae younger than 25 - 30 days (see Figure 24). After 25 - 30 days, the duration of the swimming periods increases. The duration of the passive periods decreases from about 2 minutes a couple of days after hatching to about 20 seconds at day 30. Thereafter, the duration of passive periods is between 10 and 20 seconds up to start feeding.

Light

We still do not know with certainty how the larvae react to light during the first part of the storage phase. We assume that darkness is natural for the larvae at this stage.

In the last part of the storage phase, the larvae are attracted to light. If the tank or bag walls are light/white, the larvae will keep on hitting against the wall. On the other hand, if the walls are black/dark, the larvae will, for the most part, keep to the body of water.

In connection with the last part of the storage phase in silos, some controlled experiments were carried out on the larvae's attraction to light in 1988 (A.M. Jensen and K. Naas, unpublished experiments). Larvae of different ages (276, 396, 486 day degrees) were exposed to light of various intensity and quality. It was found that the larvae reacted to light of very low intensity (< 0.5 lux) and moved towards the light source.

Experience with storage in the same silos reinforced these results. Weak reflections from the ceiling in the silo hall caused uneven and lumpy distribution of the larvae. The larvae could also be collected using light on the wall from a flashlight.

All these experiences dictate that total darkness should be encouraged throughout the complete storage of yolk sac larvae. An even distribution of the larvae could then be achieved while, at the same time, the larvae would not become stressed and unnecessarily burn energy for motion.

Stress and attack positions.

From the time the larvae are about 30 days old, it can occasionally be observed that the larvae assume a horizontal "C" shape at the same time as the tail region vibrates as if it had cramps. The larva can maintain this position for relatively long periods at a time. It always ends with the larva relaxing the "C" shape and swimming on. It is assumed that this is a stress phenomenon. It can often be observed that the larva takes this sideways horizontal "C" position when disturbed by, for example, strong light after having been kept in darkness.

76.

Some time before start-feeding, the larvae may take an "S" shape position (see Figure 25). This is an attack position which larvae of some fish species use when they want to catch prey.

Figure 25. Pike larvae (Esox lucius) attacking prey.
(From Baum, 1964).

Usually the larva takes the "S" shaped position when it finds a suitable catch. The larva elects either to attack the catch by stretching out so that it shoots forward against the prey or to release the "S" shape and swim on.

Activity and energy limitations.

During a temperature experiment, it was found that, based on yolk sac measurements, energy is not a limiting factor to growth of the larva during the first 28 - 30 days of the storage phase (see Figure 26). During this period, the larvae density may be high without inhibiting growth.

77.

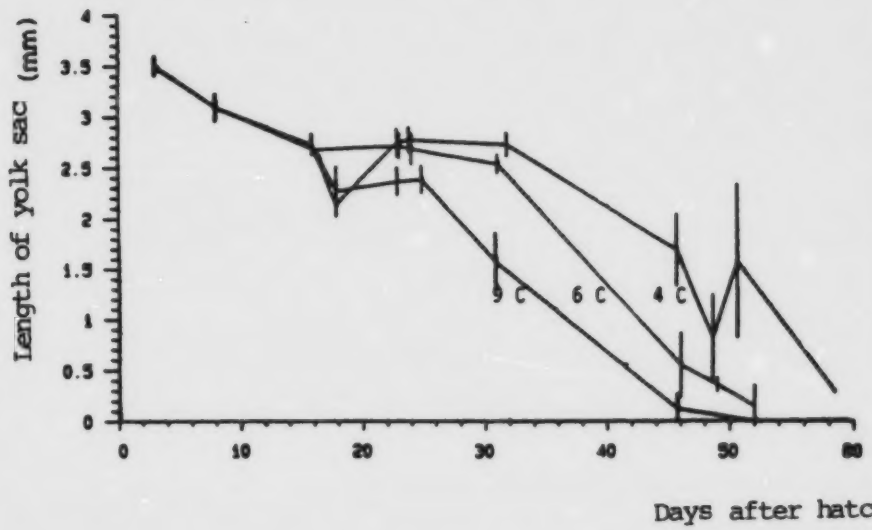


Figure 26. Average yolk sac length and standard length for halibut larvae at 4, 6 and 9 ^{o}C from day 0 until the yolk sac is consumed. (From Pittman et al, 1988).

Any increase in activity after day 28 - 30 will reduce additional growth among the larvae. Therefore, the larvae density should be low and other stress factors kept to a minimum during the last half of the storage phase.

Suggestions for solving production problems in the storage phase.

by

Torstein Harboe

and

Stig Tuene

Technical description

This chapter describes the technical solutions for juvenile production in intensive systems as they were tested during the spring of 1988 in the silo hall at Sauaneset at the Aquaculture Station at Austevoll (see Figure 27). Names of products and firms are mentioned without implying that the Fisheries Directorate recommends these products to the detriment of other comparable products.

Silo

Silos were first used in halibut experiments during the spring of 1987 at the Aquaculture Station at Austevoll. The results of these experiments were so positive that this "principle" was developed further for the 1988 season. The advantage of the silo method over bags lowered into the sea, is, among other things, better control of the water (temperature, light, movement). There are also better opportunities for tending and observation.

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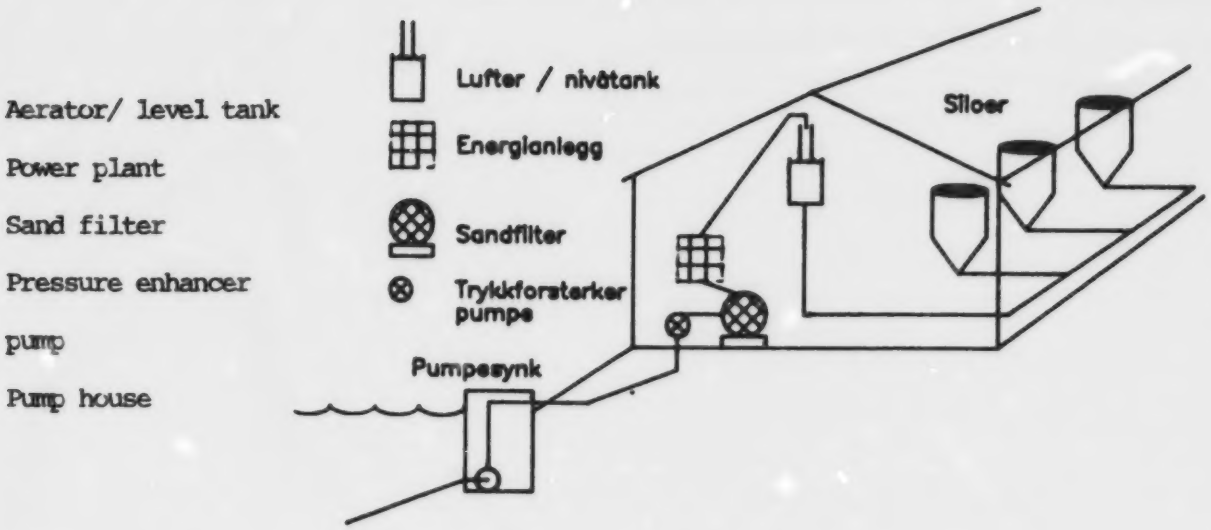

Figure 27. Existing water system attached to silo hall at Sauaneset.

Sea water.

The sea water was pumped in from about 50 metres depth. The deep water line was connected to a pumping station (Flygt, type: PSW with two pumps). Furthermore, the sea water was passed through a sand filter, at an increased presssure (3.5 kg/cm^2).

79.

The power plant

The heat pump is for use in both heating and cooling the sea water. The capacity of the plant is:

- 242 l/min. heated from 6 - 10 °C
- 200 l/min. cooled from 6 - 2 °C.

The heat pump (Type: EUW 20 F) consists of two separate refrigerant circuits which makes it possible to regulate the capacity in two stages (50 % and 100 %). Both condenser and evaporator have an external heat transfer circuit with pump and heat exchangers. The heat exchangers are made from titanium material in order to be able to withstand sea water. Glycol is used as the heat conducting medium.

Temperature control.

After the sea water has passed the power plant, it enters two collectors with temperatures of 3 °C and 10 °C. Stable temperatures were achieved by automatic mixing of water from the two collectors. The mixing valves are of the Satchwell PN 16 type with an ALX 1226 motor.

Aeration and level tanks

When heating water, supersaturation with gases occurs. All the water which was temperature treated and filtered, was aerated to avoid gas supersaturation. Two hundred millimetre pipes filled with "bio-rings" (vertically mounted) were used. From the aeration pipes the water was carried to the level

tanks. These had a constant overflow which ensure stable pressure and thereby an even supply of water to the experimental units (see Figure 28).

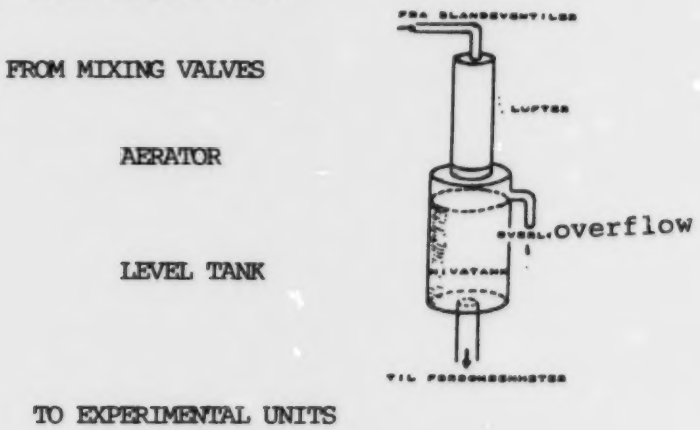

Figure 28. Outline sketch of aerator and level tank.

Regulation of water supply.

From the level tanks the water was conveyed to the experimental units. All the silos received their supply of water from the bottom, by way of a flow meter. The flow meter made it possible to record and regulate the water supply rapidly and accurately. The drain from the silos was a cylindrical strainer cup with 500 um plankton cloth. The strainer cup was connected to a flexible hose so that the drainage depth could be regulated.

Experience with silo units.

Three different types of silos were tested:

- 3 m^3 silo (6 silos)
- 5 m^3 silo (12 silos)
- 14 m^3 silo (3 silos)

Advantages

All these silos acted as they should in principle and they were well-suited for the storage phase. Windows were installed in the silos and were found very useful for observing the larvae. By being able to look in through windows and the opening in the silo cover, a good picture was obtained of the distribution of the larvae. It would have been an advantage if there had been more windows distributed vertically, particularly near the bottom of the silo. This would have it possible to observe what was happening before, during and after tending.

Disadvantages

The water flow was turbulent nearest to the water intake in the bottom. It could have been improved by mounting a distributor, "shower head", at the intake. Some of the material got stuck in the conical part of the silos, despite the sharp slope and smooth inside. This was most pronounced during the start-feeding phase.

The incoming water from 50-metre depth periodically brought large amounts of cyprinid larvae of acorn barnacles. In the silos which used unfiltered water, the water quality was very poor due to the acorn larvae which died there. This was probably the reason for the mass deaths of halibut larvae in this silo.

Operational routine during storage phase.

Tending

It is very important to tend to the silos and remove dead larvae. First of all, the decaying larvae in the system will lead to increased bacterial growth (poor water quality). Secondly, the larvae should be examined to find the possible reason for the mortality. Tending consists of removing dead material from the bottom of the silo. This was done 3 times per week. The following procedure was used during tending (see also Figure 29):

- Turn off the upwelling water in the silo.
- Add 50 litres of salted water (ca. 42 pro mille) in the bottom of the silo (see below). Use 0.75 kg fine salt to 100 litres of sea water. The salted water is heavier than the rest of the water and will remain at the

bottom. Dead material (larvae, etc.) is heavier than salt water and sinks down into the salt "plug". The live larvae are lighter than the salt "plug" and will float or swim on top of it.

- After 5 - 10 minutes the salt "plug" (ca. 30 litres) is drained off (see below).

- Turn on the upwelling water in the silo.

The placing of the salt "plug" was done by letting the salted water run from a level tank placed above water level of the silo. A hose from the level tank was connected to the water intake in the silo. The salt "plug" was introduced at a rate of about 10 l/min.. More rapid introduction could lead to the salt "plug" becoming more diluted and thus lessening its effect. 81.

When draining of the salt "plug", a hose was used where one end was raised above the water level of the silo and the other was connected to the water intake at the bottom of the silo. The intake valve was opened and the water then flowed up into the hose. By lowering the hose a little below the water level in the silo we achieved gentle draining of the water. This is important if the dead larvae are to be examined. Only 30 litres

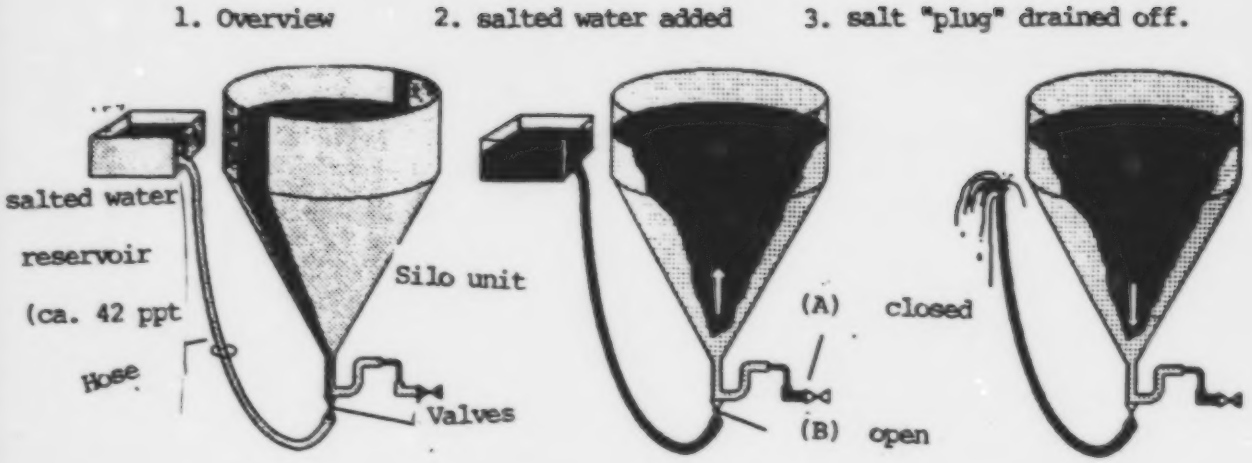

Figure 29. Tending of silo. (See text for explanation).

of the 50 litres added was drained off because most of the dead material came out with the first 15 litres. A few times, quite a number of live larvae came out during the tending and they were returned to the silo. At the next tending many dead larvae were found in the salt "plug", which indicates that the live larvae which come out with the salt "plug" will die in the course of the next few days.

Sampling

Due to the research activity, the water quality and the larvae

were sampled at regular intervals. Oxygen consumption by the larvae in the storage phase is low and is not likely to lead to critically low oxygen content in the systems described. In stagnant systems, it may be that small areas with low oxygen tension may occur around the larvae.

Dead and damaged larvae will often be found at the bottom or on the surface. These are easy to remove. If one wishes to obtain viable larvae, then they are best taken from the middle of the water column. They can be removed with a pipette early in the storage phase. Later, they are more active and must be removed using a dip net, preferably a wet net. Proper cleanliness and disinfection of the equipment is important. (See chapter on "Hygiene", page 61).

Use of light

The larvae should be exposed to as little light as possible in the beginning of the storage phase. In this phase, the light causes the larvae to swim more and consume energy they would otherwise have used for growth. The silos had covers with a light-proof inspection hatch and the window in the silo was covered with a light-proof cloth. In the silo hall, light (from flash-lights) was only used when necessary, for example, in connection with tending. If one wishes to observe the larvae during the storage phase, then the light source should

be covered by 2 - 3 layers of red cellophane. Experiments have found that the larvae do not react to red light above 600 nm (Kjell Naas and Anders Mangor Jensen, pers. comm.).

Observations of larvae in silos

In the beginning of the storage phase, the larvae are not capable of very much proper locomotion (see also "Morphology of the Larvae", page 140.) In our experiments, they occasionally settled on the outflow strainer due to the water flow against it. It was easy to return the larvae to the water by carefully shaking the strainer. This type of clogging can be limited by increasing the strainer surface or reducing the water flow through the silo. Another option is to move the strainer to an area where there are few larvae. In the first days after hatching, the larvae will float high in the silo, and the strainer can then be placed deeper down. This will, however, lead to poor circulation in the upper layers of the water.

The vertical distribution of the larvae sometimes changed. Occasionally it was found that layers of warmer/colder water had been formed in the silo. The larvae were then found in the warm water (up to 12 ^{o}C in that year's experiment). At other times it appeared that the larvae were "light" or "heavy" depending on the stage of development. "Light" larvae float closer to the surface while "heavy" larvae will float near the

bottom of the silo. At the beginning of the storage phase, healthy larvae hang head down (see "Behaviour", page 143). They do not move unless they are disturbed by light or movements. Towards the end of the storage phase they react sharply to light or movements. ("C" shape, see "Behaviour" p. 143). During this phase, continuous light resulted in the larvae beginning to swim upward in the silo. The larvae continued to swim even when they reached the surface. This behaviour could not be reversed by providing light from below. It appears that the larva orientates itself by a correlation between light (phototaxy) and the force of gravity (geotaxy)). In nature, this behaviour will help the larvae to wander up from the depths towards higher layers of water where food is available. Even when the larvae are kept in darkness all the time, they will swim towards the surface when they are about 250 day degrees old.

START-FEEDING PHASE

by

Kjell Naas,

Gunnar Nyhammer

and

Anders Mangor Jensen

Introduction

Halibut larvae live off the energy in the yolk sac until they are about 50 days old (it varies with the temperature). Nevertheless, they should be supplied with prey for some time before the yolk is consumed. The start-feeding phase is defined as the period from when the larvae are supplied with prey up to the time they metamorphose. This is the most critical phase in the production of juveniles. At present, there are two different production lines in the start-feeding phase - the intensive and the extensive system. There are conceivable and inconceivable combinations of "intensive" and "extensive", so that, in most cases, a system is not fully described by any one of the concepts.

However, it is easy to define what is meant by an intensive

system. In many contexts, it is appropriate to use this definition while, at the same time, emphasizing on which points deviation occurs.

Ideally, in an intensive system, there is control over all parameters involved, such as volume, temperature, all of the fauna (bacteria, fish, prey, and so on), light conditions, etc.. This is impossible in practice. In a non-sterile environment, the succession in micro-fauna will be almost unpredictable, which also applies to the effect of the micro-fauna on cultured organisms. Nevertheless, opportunities for monitoring in intensive systems are many and various measures are possible.

Intensive production of marine larvae has a long tradition, particularly with turbot larvae, which have been cultivated intensively since 1972. This production has been ongoing for a number of years even though, in most cases, it has provided somewhat varied and normally poor results. In recent years, increased understanding has been gained which appears to have been triggered by two main problems. Production of feed organisms (**Brachionus, Artemia**), which satisfy the larvae's nutritional needs is critical. More and more experiments indicate that the need for poly-unsaturated fat is difficult to meet through laboratory-produced live feed. The next

problem lies in incubator hygiene. An intensive system is, in contrast to an extensive one, a small buffered system with regard to micro-fauna. Opportunistic bacteria may, if conditions are right, produce a heavier growth resulting in deterioration of the tank environment, because the system has no natural preconditions for growth limitation. By comparison, an extensive system will contain considerable "natural" flora of many species. This will limit the growth of opportunists, so that the environment will stabilize.

Start-feeding in extensive systems means that the larvae are released in a predator-free system where they select their food from among the spectrum of feed organisms which are in the water (plankton). In this manner, the production of feed organisms is left to nature itself and the only "input" necessary for a completely extensive system is sunlight.

84.

For a sufficiently large amount of feed to be available, an extensive system depends on adapting operation of the system to the reproduction rate of the prey organisms. An extensive system can be described as an:

outdoor enclosed body of water where the exchange rate of water is less than the reproduction rate among the prey (or the prey's feed-organisms).

Somewhat simplified, it can be said that an extensive system has a form of "buffer capacity", that is, there are always species available within the size range that the larve graze off. This presumes a sufficiently large system, a nutrient-rich system, and that the larvae are opportunists.

In a purely extensive system, all feed organisms are self-produced in the system, as opposed to a purely intensive system, where all feed is manufactured and supplied. Between these extremes, several other models for semi-intensive start-feeding of larvae can be considered. Such an example is feeding with concentrated animal plankton from the ocean.

The first feed uptake (start-feeding) generally presents the greatest problem when trying to adapt a new species to rearing (this applies, specifically, to marine species). Feed particles must be of correct nutritional composition, have the correct taste, colour, consistency, proper buoyancy, size, and so on. The qualities of the start-feed, and the larvae's environmental requirements, will often be unknown when the rearing of new species is attempted.

With the aid of the so-called extensive method, some of the problems occurring during the start-feed phase can be avoided. The larvae are offered a broad spectrum of potential prey

organisms in an approximated natural environment and left to select which is suitable. In this manner, it is possible to discover which feed organisms the larvae prefer and the appropriate feed organisms can then be cultivated in the next round, or artificial feed of the correct quality can be manufactured. The production of juveniles can thereafter be carried out in smaller and more controllable systems.

In a closed system without exchange of water, there will also be problems with low oxygen saturation, particularly near the bottom. By agitating the water and providing a supply of silicate nutrient salts, the growth of diatoms will be encouraged. This class of algae are generally more productive than others, that is, a higher net oxygen production is obtained from a given biomass. This class of algae also contains several large and nutrient-rich species suitable as feed for large animal plankton (copepods) which, again is a feed well-suited for fish larvae. By using fertilizers and agitation, there is also an opportunity to direct the biological production in an extensive system.

85.

Intensive start feeding of halibut larvae.

This chapter describes the intensive start-feeding experiments which were carried out at the Aquaculture Station at Austevoll during the 1988 season.

Three different types of silos (as in the storage phase) were used.

- 12 silos of 5m^3 without temperature control (6 ^oC - 8^oC).
- 2 silos of 14 m^3 with temperatue control (3 ^oC and 9 ^oC, adjusted upward to between 9 ^oC and 12 ^oC in the start-feeding phase).
- 1 silo of 14 m^3 with temperature control (6^oC, not adjusted upward in the start-feeding phase).

Water flow: 10 - 15 l/min (in the 14 m^3 silos)
3 - 5 l/min (in the 5 m^3 silos).

Lighting system

The lights were turned on in the silos a few days prior to start-feeding. The light source was 36 Watt daylight tubes. The light tubes were 1.2 metre in length and hung vertically in the middle of the silo with the upper end about 40 cm below the surface. The light tube was placed in a waterproof plexiglass tube.

The larvae were exposed to light of the same intensity 24 hours a day throughout the whole start-feeding period.

Types of feed

Previously, start-feeding experiments with halibut utilized mainly collected zooplankton as feed (see Figure 30). From the 1989 season onward, collection using so-called wheel-filters will be tested.

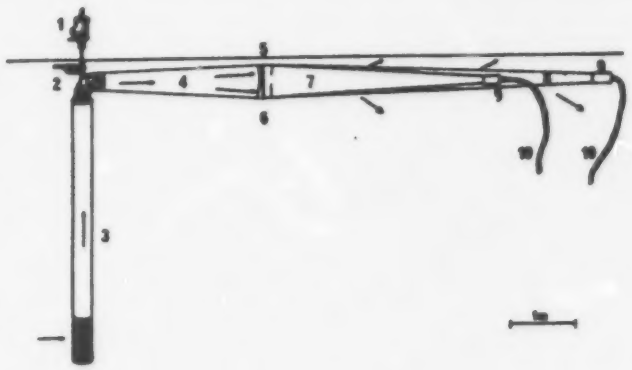

Figure 29. Example of collection system for natural zooplankton. 1) Electric motor. 2) Propeller housing. 3) Suction pipe with attached strainer. 4) Non-filtering cloth. 5) Canvas collars. 6) Zippers. 7) Inner filtration cone, (f.ex. 0.1 mm mesh size). 8) Outer filtration cone (f. ex. 0.2 mm mesh size). 9) End cap. 10) Flexible hose to the collection box. (By Kvenseth, 1985).

At the Aquaculture Station at Austevoll, two types of cultivated live feed were added to the experiment:

- The wheel animalcule **Brachionus plicatilis** (see Figure 31).
- The larvae stages of the saltwater crustacean **Artemia** (see Figure 32).

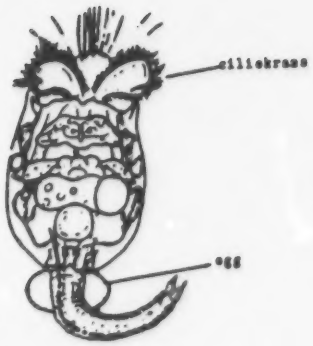

Figure 31. Brachionus plicatilis

Figure 32. Artemia

Today, these two species comprise the most used start-feed for marine fish larvae in intensive rearing. The advantage with these is that

they are easy to cultivate in large numbers. Details of cultivation and enriching are not dealt with in this paper. **Artemia:** See Læger et al (1986), and **Brachionus:** Theilacker et al, (1971). The disadvantage is that they are warm water species (cultivation temperature: 20 - 30 ^{o}C). They are not ideal feed organisms the way they are normally cultivated. Specifically, the content of long-chained poly-unsaturated fatty acids (PUFA) is low in relation to that needed by marine fish larvae (Watanabe et al, 1978). The low content of free amino acids has also been pointed out (H.J. Fyhn, pers. comm.). To improve this condition, **Brachionus plicatilis** and **Artemia** have been enriched with special nutritional particles high in unsaturated fatty acids.

87.

Different combinations of natural zooplankton, **Brachionus plicatilis** and **Artemia** were used in the various experiments. One group of halibut larvae was given only natural zooplankton.

Table 10. Approximate length of prey offered to halibut larvae in the start-feeding phase.

Type of prey	Length (mm)
Natural zooplankton	0.1 - 2.0
Brachionus plicatilis	0.1 - 0.3
Artemia (larvae stage 2 - 4)	0.5 - 0.8

Two types of single-celled algae (**Nannochloris** and **Pavlova**)

were added to the 12 silos a few days before adding these prey. Experiments have shown that other marine species, such as, for example, turbot larvae have increased their growth and survival rates by the addition of some types of algae (Howell, 1979; Scott and Middleton, 1979).

Feeding

Before being fed to the larvae, the cultivated prey **Brachionus plicatilis** and **Artemia** were cooled down gradually to prevent them from experiencing cold shock, since the temperature in the silos was much lower than in the cultivation vats. Feeding was usually done once a day, and the feed was introduced from the surface of the silo. Attempts were made to maintain a relatively constant density of prey in the silos (see Table 11).

Table 11. Approximate density of prey per liter of water in the silos in the start-feeding phase.

Type of prey	Density (prey/litre)
Natural zooplankton	100
Brachionus plicatilis	500
Artemia (larvae stage 2 - 4)	200

RESULTS AND EXPERIENCE

Lighting system

At the introduction of light in the silos, the halibut larvae swam to the surface. One great problem was that the larvae collected tightly around the light sources or light reflections at the surface of the silo. The larvae were very active and constantly collided with each other which meant that they expended a great deal of the energy which could have been used for growth. By introducing a type of brackish water (20 pro mille) combined with the lighting tube, the larvae were drawn downward in the water column. At the beginning the larvae collected around the light tube and later many of the larvae remained just below the light tube.

The distribution of prey in the silo was influenced by the light pattern. The prey are positive-photo-tactic (they move towards a light source) and the highest concentration of prey was therefore just around and just below the light tube. The halibut larvae mainly remained "grazing" just below the light tube where the concentration of prey was normally lower. It appeared that the larvae preferred the light coming from above.

88.

Water environment

Immediately after adding the algae, the total content of

bacteria in the 12 silos increased ten-fold, closely followed by a high mortality among the halibut larvae. This indicates that the addition of algae was unfavourabe in view of the number of bacteria. The light intensity in the silo was also too low to keep the algae population alive over extended periods.

Grazing activity

It was observed that grazing activity was considerably greater in the silos with the highest temperatures. A high water temperature also appears to stimulate grazing behaviour in the start-feeding phase, which concurs with results from other experiments with halibut (J. Dye, pers. comm.) and is also a general phenomenon.

The feeding behaviour of halibut larvae is described on page 164. It was observed that halibut larvae relatively often missed in their attacks on prey, particularly in the beginning. Later, the feeding success (percentage of hits) increased, particularly on **Brachionus plicatilis** and **Artemia.** The halibut larvae still quite often missed natural zooplankton which had a more effective flight behaviour than **Brachionus plicatilis** and **Artemia.** The natural zooplankton was dominated by calanoid copepods, and the oldest stages of these often jumped aside by slapping with the antennae when the halibut larvae attacked.

Survival, growth and intake of nutrient

A sharp increase in mortality was observed in the 12 silos in connection with the addition of algae just before the start-feeding. Stress from light may have increased the mortality at this time. Despite feed uptake by many larvae, most of them died after a while, probably due to hunger (too low nutrient intake). In total about 100 metamorphosed halibut juveniles were produced from 225,000 eggs placed in the silo experiments in 1988.

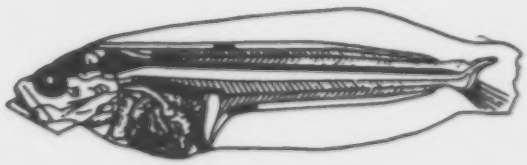

Figure 33. Start-fed halibut larva with full intestine. (The figure is taken from Pittman et al, 1987.)

The halibut larvae in the silos grew slowly when compared to larvae which were start-fed at mesocosmos (see "Extensive start-feeding of halibut larvae", page 179). This may be caused, in part, by high temperature at mesocosmos, but it cannot explain the entire difference. Good grazing behaviour was observed in most of the silos. The removal of larvae

showed that **Artemia** dominated as feed, but **Brachionus plicatilis** and natural zooplankton were also identified in the stomachs/intestines. Despite this, mortality was high. This may indicate that the nutritional content of the cultivated prey was too low (Bolla, 1988) and that the intake of nutrients by the larvae was too low to survive any length of time, particularly when seen in relation to nutritional requirements of larvae kept under stress. Natural zooplankton normally has a higher nutritional value than **Artemia** and **Brachionus plicatilis**. Natural zooplankton is not one uniform group, and composition of species, amount and size may vary widely over time and place. Suitable-sized prey may be more important than the type of prey with regard to selection of feed for halibut larvae (Naas et al, 1987). Halibut larvae have also been observed to ingest immobile particles, among others, cysts of **Artemia**, which also has no nutrional value (Dye and Brancker, 1987) and the large green algae **Halosphaera minor** (Berg and Øiestad, 1986).

89.

Semi-intensive start-feeding of halibut larvae.

Until 1987, most halibut juveniles were produced in semi-intensive experiments with the addition of captured zooplankton as start-feed. Start-feeding by this method was basically carried out by sinking plastic bags into the sea.

This produced good, but widely varied, results. The wide variations in the success is due to the fact that storage of the yolk sac larvae is also carried out in the same bags. As previously described, the larvae are very sensitive during this phase and depend on stable environmental conditions and low stress, conditions which can be difficult to meet in bags. A well-developed form of bag-rearing is described by Berg et al (1987), and the subsequent start-feeding by Naas et al, (1987).

In 1988, Sea Farm obtained very good result (close to 7 % survival from eggs to metamorphosed juveniles) using bags where the larvae were supplied with a plankton-concentrate. (L. Berg, pers. comm.). The most important prerequisite for this method lies in the opportunity to collect zooplankton from a highly productive area (a "poll" or a basin*), and that the zooplankton production is more or less stable in the start-feeding period.

Extensive start-feeding of halibut larvae

In 1988, an extensive start-feeding experiment was set up in a 3-meter-deep tank at the Aquaculture Station at Austevoll. The hypothesis was that, in a functional ecosystem, feed organisms

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* Poll = a round fjord with narrow inlet.
Basin = enclosed arm of a fjord. Tr.

would be nutritionally better than in a system with a supply of prey which could not maintain their nutritional quality by eating algae.

The tank (volume: 300 m^3) was filled with surface water ten days before the halibut larvae were ready for start-feeding. Two kilos of artificial fertilizer, having nitrogen, phosphorus and potassium as main components, were dissolved and added to the tank during filling. This produced a theoretical nitrate concentration of 30 uM. In the days leading up to placing of the halibut larvae, zooplankton of 240 - 500 um in size were collected and added to the tank.

Throughout the yolk sac phase, the halibut larvae were stored in a 15-metre upwelling silo at about 4 ^{o}C. On day 51 after hatching (238 degree-days) between 3,000 and 5,000 start-feed ready larvae were transferred to the tank.

During the experiment, low oxygen values were not recorded anywhere in the tank. This may possibly be because the water column was so shallow that the water was within the lighted zone. The depth of the tank will therefore be of great signifance to water quality and probably should not exceed 4 metres.

90.

The intestinal contents of the larvae corresponded to the

composition of the prey. Only copopdites (800 - 1,000 um) of harpactoid copepods were found in the intestines (8 larvae examined).

On day 100 after hatching (907 day degrees) 150 fully metamorphosed halibut juveniles were collected by light during the night and the next two days the remaining survivors (159) were collected by lowering the water level. The juveniles which had shown phototactical response were somewhat smaller and not as pigmented as the remainder, which were at the bottom. The results show that halibut juveniles can be collected after metamorphosis by using light and that it should be done at 800 - 850 day degrees.

In this experiment, the population of halibut larvae was probably never close to the system´s carrying capacity. If, in a tank of this size, one wishes to produce a reasonable number of juveniles per unit, then it can be assumed that the carrying capacity would be exceeded. The strategy must be to add prey progressively until the juveniles are big enough to be weaned to artificial feed. In such a large outdoor tank, it is nevertheless expected that the primary production would be sufficient for even the added prey to be well-fed and nutrient-rich at all times.

FROM METAMORPHOSED LARVA TO JUVENILE

by

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Anders Mangor Jensen

and

Anne Berit Skiftesvik

Metamorphosis actually means rapid conversion and is probably an imprecise expression in this context. Nevertheless, it is a practical method of describing the larvae's development because, particularly for flatfish, there are clear morphological indications that the larva has become a juvenile.

The practical aspect of this is that the larva after metamorphosis is far more robust than a larva proper. Among other things, it can be dip-netted and transferred. In the case of some of the species, it can also be directly weaned to artificial feed. The metamorphosis among halibut is completed when the juvenile has obtained the characteristic appearance of both eyes being on one side. This is in contrast to cod, for example, which has a gradual transition to adult fish.

Larvae in the storage phase have the eyes located on either

side of the head. The left eye begins to migrate towards the right eye some time after the halibut is first fed.
It swims all the time in a position which keeps both eyes in a horizontal plane. From having one eye symmetrically on either side of the head and swimming as do other fishes, the halibut begins to twist its body more and more towards the side when it swims, as the left eye migrates towards the right side of the head. (This is general for all flatfishes).

Figure 34. Metamorphosed juvenile (ca. 90 days).

After metamorphosis, the halibut settles on the bottom. Now it is active when searching for food, when eating and when searching for a resting place. Otherwise it is quite passive and "presses" against the bottom. It has a great capacity to camouflage itself. If the bottom is sandy it may make a resting depression and it often digs itself down.

The halibut juvenile has a certain degree of agressive behaviour towards its own species. Large juveniles, for example, chase small juveniles away from the "best" depressions. Due to competition, halibut should be sorted according to size. It is also common to size-sort the juveniles so that the smallest and youngest can still be fed with collected plankton.

Juveniles which have metamorphosed can be kept in flat-bottomed tanks with 100 fish per m^2 as long as they are smaller than three to four centimetres. They can also tolerate greater densities.

92.

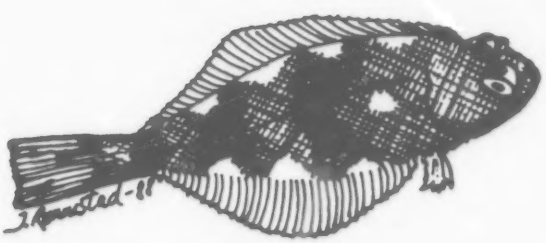

Figure 35. Juvenile - about 4 months old.

When moved, the fish becomes stressed and can easily be

infected by vibriosis. In 1987, the juveniles at the Aquaculture Station at Austevoll were attacked by vibriosis and only 25 of 200 survived. When vibriosis is suspected it should be treated with Imiquil, for example. (See under "Disease").

Feeding the juveniles

The halibut larvae will metamorphose about 90 days after hatching. They will settle on the bottom some time later. The larvae are adapted to dry feed (weaned) as soon as possible after settling at the bottom. (See Figure 14, page 120). This can be a difficult and time-consuming process and may last more than one (1) month. To a large degree, a mix of calanus finmarchicus and wet feed has been used during the initial period. Calanus finmarchicus spoils very easily and must be brought out frozen (the freezing technology is also important). It also contributes to some pollution of the water. The type of wet feed used is standard dry feed (see below) to which water has been added and which has been pressed through a garlic press to obtain the right diameter.

It is possible that the juveniles can be weaned directly to dry feed without first being used to calanus finmarchicus feed, but due to the small number of juveniles, this has not been tested in controlled experiments.

For various reasons, there will always be some mortality during the weaning period. Weaning success among turbot has been shown to be dependent on the size of the larvae, previous feed uptake and composition of the weaning diet (Paulsen, 1987). The most important factor is the quality of the live feed before weaning. These conditions have not been investigated in detail for halibut, but it has been observed that some of the weak juveniles never eat any dry feed and finally die.

In the beginning of Septmber 1988, the temperature at Aquaculture Station at Austevoll increased from 9.5 ^{o}C to 13 ^{o}C. This led to a marked increase in appetite among the 1988 generation. On the other hand, reduced appetite was observed among the 1986 generation. This correlation has been described for many species.

In contrast to turbot, for example, the halibut juvenile does not particularly search for food and will lie on the bottom a large part of the time. The feed must almost drift by its snout in order for the juvenile to take it (G. Nyhammer, pers. comm.).

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Fry up to 100 grammes can be hand-fed several times a day or an

automatic feeder may be used. Nowadays it is common to feed the halibut every second day after a long transition period. This has not been systematically investigated.

Observations at the Aquaculture Station at Austevoll have shown that the juveniles prefer a white/light pellet to a dark/brown. This observation has not been systematically investigated and may be background-dependent. The juveniles also appear to prefer large pellets of 1.5 mm size compared to the smaller 0.5 mm. The juveniles were between 2 and 7 cm at the time of the experiment.

Halibut juveniles which had been weaned to dry feed grew well on that (T. Hjertnes, pers. comm.). The percentage of growth varied between tanks and between individuals. The variation may have been due to insufficient knowledge about feeding routines. The composition of the feed was probably also not ideal. The composition of the dry feed used was identical to that used for halibut food fish at the station (see "Feed and Feeding", page 47).

The juvenile is considered marketable after metamorphosis when it has been weaned to dry feed.

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APPENDICES

Appendix I

Vocabulary

Algae: Types of plants which grow in water or close to water. The size varies from single-celled to micro-algae (i.e. Crysochromulina polylepis) to large macro-algae such as kelp and sea tangle.

Amylase: An enzyme which breaks down carbohydrates (sugar,etc.) so that it can be absorbed in the intestine.

Copepod: A type of crustacean such as, e.g. **Calanus finmarchicus.**

Cryopreservation: Method of freezing and storing milt.

Domestication: An individual and genetic adaptation to captivity.

Egg quality: Egg quality can be described as the eggs' ability to produce viable juveniles. Egg quality is determined from the moment the egg leaves the mother fish and fertilization has taken place, and the effects of later treatment will not be taken into consideration.

Extensive system: An outdoor enclosed body of water where the rate of exchange of the water is lower than the reproduction rate of the prey (or the prey's feed organisms).

Exogenous feed uptake: Uptake of nutrients which do not originate from the individual itself.

Uptake of the yolk mass is endogenous feed uptake.

Extruded feed: A production process for feed mixes where steam and overpressure are used. This treatment has been found to increase digestibility of carbohydrates by breaking long chains into smaller ones. The feed will have better buoyancy than conventionally produced feed, due to incorporated air bubbles. Further, there will be greater opportunity to manipulate the content of fat in the feed.

Ensilage: Here used for fish ensilage. Ground fish raw material (normally with antioxidants added to prevent rancidity) which is stored in acid. In Norway, formic acid is used (acts as bacteria inhibitor). For long-term storage, sorbic acid is sometimes added to prevent growth of mould.

Feces: Manure or feces from fish.

Fecundity: The capacity or ability to produce progeny.

Follicle cells: Cells which surround the egg cells.

Gonads: Ovaries or testicles.

Hydration: Water is supplied or added, as opposed to dehydration (loss of water).

Iris: The diaphragm aperture which regulates the amount of light which reaches the sensing cells in the eye.

LT meal: A high-quality meal which is dried at low temperature. Thereby, greater accessibility to individual amino acids is achieved.

Metamorphosis: Rapid conversion from larva to adult form.

Morphology: Structure or form of an organism.

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Soft pellet: A pellet made from a feed mix which has a dough-like consistency. The feed mixes are normally divided according to water content. Wet feed contains about 70 % water, soft feed between 40 and 50 % and dry feed about 10 % water.

Myotome: Muscle segments or the origin of these.

Notochord: A lengthwise structure of cells which is the source of some parts of the vertebrae.

Convertible energy: The amount of energy in the feed which is available to the fish for growth and maintenance of the body tissue, activity and production of sexual products. This is not equivalent to all the energy in the feed because one part is lost as fertilizer and urine.

Oocyte: Egg cell

Opportunist: Here used about a specie which bases its reproduction strategy on large amounts of eggs with no parental care of juveniles and which eats what it finds in its natural environment.

Ovulation: Freeing of eggs in the gonads. This is the time when the fish is "ripe for stroking."

Pathogen: Disease producing.

Pellet: English word, also used in Norwegian, of an oblong round feed particle - as opposed to granule.

Pers. comm.: Personal communication. In scientific papers this is a normal method of explaining that the information was obtained in conversations and has not been published.

Plankton: Organisms which live in oceans and seas and which float passively or have limited locomotion.

Population: Groups of individuals having mutual reproduction, and which are reproductively isolated from other populations.

ppm.: Parts per million (millionth, which is the same as mg/l).

ppt.: Parts per thousand (thousandth, pro mille).

Vitellogenesis: Formation and concentration of nutrients in the egg.

Vitellogenin: Nutrient which is produced in the liver of the female fish and transported by the blood to the egg.

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Appendix III

Treatment of sick and dead halibut.

by

Odd Magne Rødseth

and

Ivar Rønnestad

If, for unexplainable reasons, the fish begin to die, it is important that the necessary samples be taken so that the disease can be identified. Agreements must be made with a pathology lab, veterinary office or the like, which can deal with the collected samples. At the Aquaculture Station at Austevoll, an autopsy procedure has been prepared; it is shown here. To follow this, some knowledge, training and equipment are needed.

After completing the procedure, the remains of the fish are placed in a disinfectant (chlorine solution or formic acid) before it is destroyed or buried. Disinfection and destruction of dead fish must also be carried out on fish which have not be autopsied.

Fish to be examined should be dying or recently dead.
Fish which have been dead for several hours are of reduced diagnostic value.

Procedure:

1. Anamnestic information concerning behaviour of the fish which may be of use during the diagnosis of the disease.

   Note:

   Swimming motions:

   Breathing frequency:

   _____

   Pigmentation:

   _____

   Appetite:

   _____

   +/_ hook in throat and esophagus

   _____

   Other

   _____

   _____

2. Kill the fish by bringing a scalpel or knife down between the brain and the first vertebra.

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3. Decide whether the fish can be examined immediately. If this is not the case, then it must be placed on ice or frozen once steps 6, 7 and 8 have been carried out.

4. **AUTOPSY**

Date: _____

Hour: _____

Tank No. _____

Fish No. _____

Length: _____

Weight: _____

5. External pathological changes/ectoparasites.

Visual changes (Pigmentation, bleeding, etc.).

_____

Mucus (slime) amount

_____

Condition of eyes

_____

+/- Halibut fluke

_____

Wear on fins

_____

Other (e.g. bloody tail)

_____

6. Cut off gill cover, cut off parts of gill arch. Formalin fix at a ratio of 1(sample) : 10 (neutral 4 % formalin).

7. Gill scrapings. Keep refrigerated. For microscopy.

8. Skin scrapings. Refrigerate. For microscopy (for Trichodina sp. and Ichthyobodo sp. (Costia)).

9. Open the fish to expose the organs.
   1. cut: from anus and to head.
   2. cut: from anus and angularly up to lateral line and forward to the head.

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10. Pathological organic changes. Observe and note:
    Bleeding

    _____

    Colour changes

    _____

    Growths/granuloma

    _____

    Accumulation of fluid in the abdominal cavity

    _____

    Content of stomach/intestine

    _____

    Swollen kidney, spleen

    _____

    Appearance of heart/heart sac

    _____

    Gonads

    _____

11. Bacteriology. Incise the kidney with a sterile scalpel. Put a cotton swab or sterile hypodermic needle in the kidney. Rub on agar (blood agar and/or Tryptome soya agar). If strong suspicion of bacterial infection, then the sensitivity to antibiotics should be tested on primary isolate.

12. Blood sample. Centrifuge immediately.

13. Tissue samples 1 cm^3 from histologically interesting organs:
    Fix in 4 % neutral buffered formalin.
    - liver
    - heart
    - kidney
    - spleen
    - pylorus (appendix)
    - intestine
    - gonads

14. Remove hypophyses

(15. remove gonads)

16. Tissue imprint from kidneys and spleen is excised. Remove excess blood by pressing the tissue piece against a filter paper. Press the tissue piece lightly against a glass

slide, 3 imprints per glass slide, a total of 4 pieces.
Air dry preparation and colour with (M+D Diff- Quik) -
microscopy.

106.

The following equipment must always be in the laboratory:

Binder with blank forms
Cooler bag
Plastic bags
Glass slides
Sterile cotton swabs
Scalpel
Scalpel blades
Tweezers
Bone scissors
Test tubes (for tissue samples)
Blood agar
Tryptone soya agar
Vibriostatic plates
Waterproof India ink
70 % ethanol for cleaning scalpels

In ventilated cupboard:

Bottle with Formalin, neutral

Formalin, neutral, (1 litre ready-mixed)

114 ml 35 % formalin

886 ml H_2O

4.00 g $NaH_2PO_4{}^*2H_2O$

8.15 g $Na_2HPO^*H_2O$